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ABSTRACT

This report focuses on the open laboratory procedures used in the freshman biology course at Hamline University. The study aimed to review a number of specific methods used in conducting the unstructured and unscheduled laboratory procedures and discover the most effective arrangement using those procedures. The report includes detailed discussions of methods used in the open laboratory. Problems of equipment storage, clarity of directions, laboratory assistants, faculty responsibility, conference periods, examinations, record-keeping and report writing are examined. Comparisons are made between student time spent in the laboratory and grade performance, and between space utilization in the traditional laboratories and in the open laboratories. Final forms of laboratory manuel revisions and results of student evaluation forms are included. The conclusions concern faculty-student relations and their reactions to the course. It is noted that although the open laboratory itself was successful, the general biology course was cancelled and replaced with separate courses for majors and non-majors. The last section of the report contains the rationale and outline of the new biology curriculum resulting from this study and includes methods for continuing evaluation of its content and effectiveness. (DS)

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Evaluation of, and Revision of Open Laboratory Procedures at the College Freshman Level

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July 1969

U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
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SUMMARY

This study, Evaluation of, and Revision of Open Laboratory Procedures at the College Freshman Level, was carried out over a period of five years with the thought that its results would help solve some major difficulties in General Biology instruction. Its objective was to revise and review a number of specific methods used in conducting Open Laboratory in our two-term sequence, in order to discover the most effective laboratory arrangement using this method. The report contains copies of the final form of the laboratory manuals written and used, Open Laboratory 1 and Open Laboratory 2, and therein outlines that effective arrangement.

Rather than continuing the course at the end of the study, the biology faculty at Hamline University decided to cancel General Biology entirely, because of difficulties inherent in General Biology itself which the use of the open laboratory helped to identify, and substitute a course with restricted objectives for liberal arts students plus a six-term biology core curriculum for biology majors.

Detailed discussion of methods used in the open laboratory are included in this report, so the interested reader may decide on their merits whether or not to adopt this method himself. If his major concern is saving space, he is urged to consider this as a valid alternative to mechanized demonstration laboratories, and the relative merits are discussed. Problems of clarity of directions, storage of equipment used, laboratory assistants, faculty responsibility, conference periods, record-keeping, report writing and examinations are presented. A comparison is made between the space used with this method and with traditional scheduled laboratory sections, pointing out the large savings that can be made.

The numerical part of this report is concerned with time cards that students kept of the intervals spent in the laboratory, and each student's total number of minutes spent per term is compared with his laboratory performance. Although the data are not presented with claim to high accuracy, a tentative conclusion is drawn that those who spent more time in the laboratory achieved higher scores. Some further observations concerning the time cards are made, that are not apparent from the raw data; that the system used is flexible and allows the student to assign his own time, make up his own deficiencies at his own discretion, and do that in segments of time that are most effective for him.

Results of laboratory manual revisions are included only in the final form of the published manuals, since the several intermediate versions were not thought important enough to quadruple the bulk of the report. Results of part of one year's student evaluation

forms are included, as well as a facsimile of that year's entire questionnaire, to show one of the most important instruments used in making changes in the course and revisions in the directions.

Conclusions are drawn with respect to faculty reactions to the course, and their responsibilities, lectures and interrelationships. Factors concerning student reaction to the course, their saving of time and their behavior in the laboratory are presented. The question of a General Biology course is raised, and the arguments of the Hamline University Biology faculty are reviewed that led to cancellation of the course. The primary reason given is that such a course tries to do too much, and it cannot do what it professes to do in two terms without either overload or omission of important portions. Their solutions to the problems of too much work for non-major students and superficiality for biology majors are presented.

The section on Recommendations is made up primarily of a rationale and outline of the new biology curriculum that arose in part as a result of the study of open laboratories. It further recommends that when new curricula are adopted in biology, at the same time machinery be set up for continuous evaluation of content and effectiveness.

INTRODUCTION

This study of open laboratories was carried out over a period of five years with the thought that results of it would be helpful in solving some major difficulties in General Biology instruction. The overall objective was to produce a course of study at the college freshman level that would reduce repetition and increase independence of freshman students. In making the request for support we felt that, despite the explosion in the volume of biological information, it should be possible to produce a two-term sequence that would cover the important principles in biology, demonstrate these principles while introducing the students to the most pertinent laboratory procedures, give experience in how a biologist works in the laboratory, how he keeps records and writes reports. In sum, we constructed a single two-term course to prepare the potential major for more detailed study in later courses in the department, and to give a broad biological education to liberal arts students from outside the department.

Rationale

The center of this study was the Open Laboratory. It was thought that students were coming to college with such diversity of knowledge (BSCS biology, traditional biology, second courses in biology, etc.) that many of them were wasting a great deal of time attending structured laboratories of determined length in which a particular set of activities was outlined for the day. They were taking up space while repeating many procedures previously performed, and meanwhile were missing important laboratory procedures and concepts that they might otherwise be studying. The Open Laboratory seemed to be the ideal solution: the student would be provided with weekly directions clearly understandable without further explanation, he would come to the laboratory choosing his own time, go quickly through those things that he had already done or already understood, and this would give him time to spend on unfamiliar facts and procedures.

Each student in such a course would come out with a great deal of knowledge added to his store, and the only students who would be penalized would be those few who had no biological background at all. It was assumed that there were very few who entered college biology who had not already had some biological study, either in junior or senior high.

Our assumptions that there is a body of knowledge, that it contains most of the important principles of biology, and that it can be covered in two terms, followed common practice in colleges that offer a beginning biology course.

Primary Objective

Our primary objective was to evaluate what we had already done, and then to develop the laboratory directions and their concomitant procedures.

Background

General Biology at a college of this size is usually considered to be a two-term sequence open to majors and non-majors without prerequisite. Even if the college regulations provide a choice among the various introductory science courses to satisfy a science requirement, students normally choose biology, because it is considered to be the most understandable of the sciences. This popular opinion unfortunately ignores biological development over the last forty years, the understanding of which first requires an understanding of chemical and physical principles in some detail before the student can understand the biological principles. Biology departments who present General Biology to meet the popular desire for an "understandable" science course must therefore limit what they teach to a very small selection among the many biological topics.

If any of the students in the course lack some basic biological topic, then that topic must be covered to some extent, no matter how many students have already had it. Such repetition in lectures may be advantageous, but required repetition in the laboratory of elementary procedures usually can have little advantage, and is actually harmful if it cuts into the time a student ought to use in learning more advanced procedures. A student initially bored is difficult to interest when something new finally does appear. It is more difficult to rekindle ambition in a student who repeats or omits routine business at the first of a course, than it is to start him out with something that is new to him. The element of choice in Open Laboratory was designed to avoid this problem; each student concentrated on his own deficiencies.

It is a rare college that requires entering biology students to have chemistry as a prerequisite in biology, and therefore biologists have not felt that they could require chemical techniques of their students in biology. This tends to reduce the scope of the general biology laboratory to microscopic observation, dissection and taxonomy. We chose to teach the necessary chemistry instead: time saved in Open Laboratory was used to get students used to chemical terminology, reactions, and point of view.

Due to the vast numbers of students taken into most college General Biology courses, it has been thought impossible to teach many up-to-date laboratory techniques that require expensive instrumentation. It was thought that open laboratory would aid in instrumentation problems, by efficient use of fewer instruments over a longer period of time.

With reference to the background of students, such a course takes in a highly heterogeneous population, and intends to make of them a group that is uniformly educated in the biological sciences, or at least in biology as conceived in the past. In order to take best advantage of the past background of students in such a course, it would be necessary for a college to set up and operate a large number of sections, each with its own approach and subject matter. Most colleges do not have the space or the faculty to permit this.

Our hypothesis was that, properly conceived and executed, open laboratories should be able to help solve the difficulty of background. Students would be made responsible for a large amount of laboratory material, but would make their own decisions about what work they still need to perform and what they had already learned in the past, and they would be allowed to set their own time during the week when they would do their work.

Previous Conclusions

In the first two years of the study, before the grant was requested, we had concluded that the following items had already been accomplished and further work would lead to their improvement:

1) Repetition of work was avoided.
2) Tying up the laboratory with scheduled sections was avoided.
3) Students who had need of more time in the laboratory would always have the laboratory available for their use.

Our question, "Will Open Laboratories work at the freshman level?" was answered in the affirmative before the study began, given certain procedural modifications which the study was designed to find and make. In addition we had already determined that the following were possible:

1) To make materials available to a student during an entire laboratory week, with living cultures alive and in good condition.

2) To store materials so students could return to them easily.

3) To check out certain materials to the students for the full duration of the course.

4) To make directions that are clear enough for the students to follow in an open laboratory.

5) To get along without traditional "lab-lectures".

6) To make a laboratory manual illustrated with half-tone

photographs showing laboratory procedures.

Original Goals

It was planned that the study would achieve a number of goals, and some were achieved more successfully than others. We wanted to evaluate the data gathered in the first two years, reorganize the weekly laboratory schedule to distribute the load more evenly, eliminate unproductive laboratory work, improve wording and arrangement of laboratory directions, produce half-tone illustrations, and produce 8mm technique films. The last objective was postponed because many good commercial ones were released during the study and therefore producing our own was inefficient.

Additional Goals

It should be made clear that there was a lecture-theory portion of this course, not a part of the supported study, which nevertheless is partially covered in this report. Many different experiments were made with lecture techniques to find their best arrangement in order to coordinate them with the laboratory schedule, to improve the quality of all visual aids, and to find the most effective means of participation by department members. Thus certain aspects of that study will be included in these results.

Other matters are also reported under Results herein that were not a part of the original objectives of this study, but which came up during the study, such as laboratory hours, assignment of assistants, evaluation of students' background, etc. It is hoped that this inclusion will aid the reader in understanding the total program.

Value of the Study

We hoped to be able to make a contribution to biological education in general, by reporting an example of how the open laboratory could be made to work. This report may be read in that light, since we did do that in fact. The conclusion that we reached, however, after five years of study, is that the concept of General Biology is basically wrong; that it is fraught with so many obstacles that it should not be taught at all.

For other people, the value of this report may be that it points up the problems that we encountered and the solutions we discovered in making the open laboratory work, but the value of the study to us was in determining more clearly what students in biology ought to be taught, and how rapidly and thoroughly it should be presented. We put the highest possible effort into teaching the course described herein, and as a result of that effort, it worked well. In thus functioning successfully, it demonstrated better to us than any other method could have, the weaknesses of the General Biology approach to biological education today.

This course was a necessary part of our evolving curriculum, and other colleges may need to follow the same route in order to arrive at a full Core of biology courses such as we now have. The Core program that we have evolved would not have been possible without this experience in the open laboratory.

Initiation, Success and Cancellation

Hamline University adopted a 3-3-3 calendar in 1963, and the impact was an important one for all departments, since a part of the adopted procedure was the levelling in value of all courses to a single "one term course" credit. The term course is equivalent to 3 1/3 semester hours or 5 quarter hours credit. We maintained the requirement of two science courses for all students, but the value of these courses dropped from 8 or 10 hours to 6 2/3 hours. Before the 3-3-3 was adopted, the standard course outside the sciences was the three-semester-hour package, but in the laboratory sciences, courses were usually 4 or 5 semester hours in value. Outside the sciences the courses had to increase by 11% to achieve the goal, while in the sciences a 17% to 33% cut was necessary to gain the same end. Since at the same time the length of the term was cut from 15 to 10 weeks, there was probably more pressure to cut than to increase.

The science faculties were restricted by the nature of their courses and sequences to include certain ideas and techniques in the structure of the courses. The 5-semester-hour General Physics course went to three terms, and covered the same material in about the same time, but in the 4-hour chemistry and biology courses the result was accelerated courses with much the same content as before, but with 17% less time in which to cover it. The sequential nature of the General and advanced courses in chemistry, and the dependence of biology on chemistry, seemed to demand this course of action. There was therefore a great deal of pressure in both departments to eliminate unnecessary and repetitive procedures and contract laboratory time.

An additional pressure on biology at the time was that the Nursing program was dropped at about the same time as the above, which changed the character of the courses radically. Numbers of students in the courses decreased, and the emphasis of the department became modified toward a greater emphasis on true biology majors and preparation for graduate school.

In the light of these pressures the Biology Department reassessed its curriculum. We decided that although satisfactory from many points of view, we needed to break loose from the pattern because much of it was losing productivity. Previously the four members of the biology faculty at Hamline were spending much of their teaching time in introductory courses: the department offered one year each of General Botany, General Zoology and General Biology. These and the upper-division courses were offered with little plan of prerequisites and much time was lost in repetitious introductions. We decided that the biological principles and theories in the various sub-disciplines had become enough alike so that a single two-term course could be taught as an introduction to all the fields, taught both to non-majors and major students, and it would be the prerequisite for all other courses in the department.

This course was made the responsibility of a single member of the faculty, but shared by all members in a loose team-teaching arrangement. There was only one lecture section, the group was divided into 16-to 20-student conferences, each under a single faculty member, and the open laboratory became the joint responsibility of all faculty members. Using this format, we planned to release the other faculty from full responsibility for separate beginning courses, and they would have time to concentrate on and improve efficiency in their upper division courses.

We left the eventual fate of this General Biology course open: it would either succeed and continue to be our introductory course, which seemed to be the most likely outcome, or it would fail and be replaced by some then-undetermined sequence. In actuality, it succeeded and was replaced, because of its success. A review of the amount of time spent in the laboratory by the B-or-better students shows the overload that such a successful course places on the conscientious student. The same overload was reflected in the faculty.

In summary, the study intended to discover the most effective laboratory arrangement, and we believe we discovered that arrangement, and through it produced a general biology course that worked. However, because of difficulties inherent in General Biology itself rather than in open laboratory, at the end of the study we decided to abandon the course, and substitute a Biology Core that would better solve the problems as we saw them at Hamline University.

METHODS

Identification of Student Background

Although it was not included as a part of the request for funds, we had hoped to be able to run a correlation study between the kind of background that the student brought to the course and his grades in various aspects of the course. In 1966-67 a simple questionnaire was prepared (See Appendix 1) which we felt would give us adequate information for our needs. Its results were inconclusive, however, because 70% of the students could not remember what kind of high school biology they had had, and commented that they felt it made very little difference anyway since most had taken the course in the tenth grade and they could not remember much from that long ago.

In 1967-68 we tried a more sophisticated approach (see Appendix 1). As in the previous year, nearly 60% of the students could not remember what their high school course had been like, but of those who could remember, there were mixed responses and comments. The comments seemed to add up to two conclusions, neither of which has much to do with this study. One is that high school teachers seem to teach in several different ways during the year, and one thing many include is some study of living things in depth (for example, in scheduling one of the BSCS laboratory blocks). The other conclusion is that many teachers appear to use BSCS publications as texts, but continue to handle the laboratory in a much more traditional fashion than one might expect. Apparently, at many schools, the BSCS publications were adopted as a new text rather than as a new point of view in biology.

Since this kind of questionnaire seemed to be impossible to prepare unless we went into so much detail that an entire new study would be required on this subject alone, we decided to try a cross-section interview by the Chairman, to determine the general quality of the high school course. This broke down early in the interviewing process, because the Chairman found that he was assigning the high school course to an arbitrary rating scale on a completely subjective basis.

Since this was not a part of the study we decided only to report the attempt, with the hope that someone will carry out such a piece of research as an aid to sectioning college freshmen in the future.

We wish now that we had studied the chemistry background of those students; which would have probably been easy, but which did not seem to be particularly pertinent to our own study. At the present time we have the feeling that the chemistry background of students is much more pertinent to their eventual behavior in college biology courses than the biology background is, but we do not have reliable data from our own courses on this. Nevertheless, we have built our new curriculum around a chemistry prerequisite for biology majors.

Arrangement and Organization of the Laboratory Room

Our use of the Open Laboratory required one separate room open Monday through Friday 7:45 a.m. to 5:30 p.m., and for this course we chose the largest but least well-equipped of the laboratories in the department. It seated thirty-two people at four long eight-place tables, each station with four locked drawers and one unlocked door. During one term we handled 157 people easily, and could have handled at least 30% more without crowding.

The lockers (drawers) were assigned to the students, most of them singly but some shared. Behind each of the doors we kept one compound microscope with 10x and 43x objectives, a good microscope light, a dissecting pan, and when necessary the boxed set of slides being used at the time. Since the room usually was staffed when open, we felt justified in keeping these doors unlocked. Losses over a period of 5 years involved one dissecting pan and two sets of slides of twenty-five each. A few slides were damaged individually, a few clips were removed from microscope stages, and there was occasional mixing of the numbered items from one space to another, but our problems were minimal in this connection. We had more trouble with general messiness than with anything else, because the students had only assigned lockers, not assigned seats.

At the beginning of each term, we set up one day to check into the laboratory and at the end of the term another day to check out of the laboratory (see Appendix 2). We arrived at the locker list after several years of trial. Other than actual breakage, no laboratory fee was charged the students.

At the beginning of this study, our greatest concern was that we would get too many people in the laboratory for the number of work stations we had, and indeed this was true during the first two or three weeks of each fall term. We made rather elaborate precautions to avoid this: that each student when he came in would give his laboratory card to the assistant in charge and that as soon as the room became filled with thirty-two people the assistant would be aware of it and would lock the outside doors. This proved to be unnecessary and we dropped this system, simply leaving the cards in the wall rack.

Students who first came at crowded hours tended to readjust their own personal schedules and would come to the laboratory at times when there were fewer people there. One could go into the laboratory at any time during the day and find between twelve and fifteen students working. This would be true on any day except practical examination day, when the crowds became somewhat larger.

The laboratory tables contained only electrical outlets, without water or gas. There was a sink at each end of the room which the students used for washing glassware, and this was reasonably satisfactory. The problem of gas we solved by equipping each table with two propane torch bunsen burners, and this was usually adequate. We had considered purchasing alcohol burners for each locker , but it never seemed necessary. New propane cylinders were expensive, but considerably less costly than installing gas jets in each table.

There was a front demonstration table and a longer counter on one entire side of the room under the windows. This was extremely important, because all of the general space was needed for the storage of materials used during the week and later for the storage of the microcommunities studied over a period of time. A set of display cases at one end of the room was almost completely useless for this course, since we used very little demonstration and display material. One set of sidewall cabinets contained the binocular dissection microscopes that the students used occasionally throughout the course, but did not need to have immediately available. The room contained two large blackboards which were useful for temporary instructions. A standard household refrigerator was an important addition in which to keep materials the students needed to use, but which would spoil on standing unrefrigerated; such as agar plates, foodstuffs to be tested, egg albumen, etc.

Probably the most difficult storage problem was that of chemicals and cultures that the students needed to have at hand instantly, but which were needed in large amounts. For example, Laboratory Week 2 needed a large number of materials to be tested and a larger number of reagents to be used. We solved this by using dropping bottles: clear ones for materials to be tested, and brown ones for reagents. We kepts sets of these in plastic plant trays and put a set of each at each end of the laboratory tables. In the case of large numbers of students working in the laboratory at one time, this was not perfectly convenient, but it worked out quite well. We kept extra amounts on the side table so that when a bottle became empty it could be refilled by the student immediately. We attempted to do much the same kind of thing with cultures of Protozoa, fungi, and algae, but this did not seem to work as well. Since the students made microscope slides of these, we decided it would be best to keep culture dishes of these under binocular microscopes on the side table and the students could go there, make the slides, and then return to their places for examination.

We used boxes of fifty different microscope slides at each station. Although each box was numbered, and each slide was labeled with the set number and the slide number there still was a considerable amount of mixing among slide boxes. Students did not feel a sense of responsibility toward their slides, and many days would end in a state of complete confusion with numbered slides on microscopes, side tables, and student tables. If we were going to continue with the open laboratory, we would have to devise a better method for storage of slides.

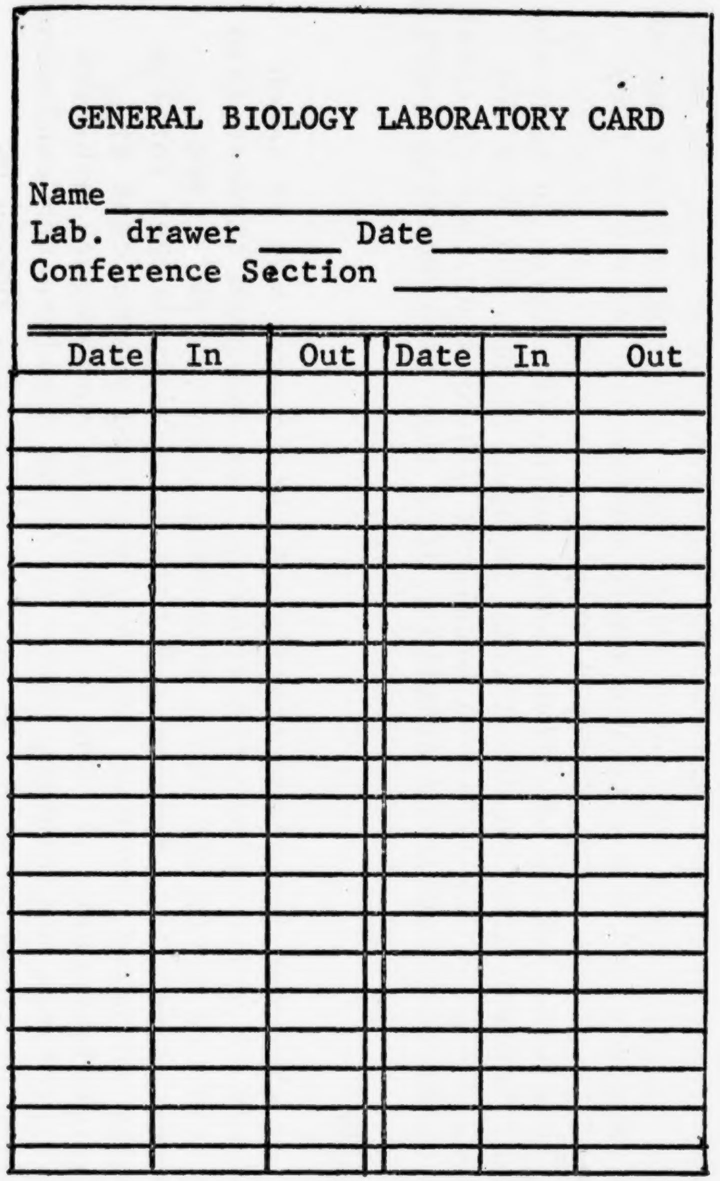
Laboratory Assistants

We expected our assistants to work for five periods per week, but scheduled them for only three periods per week. One of the extra hours was used during heavy times when they were responsible for the laboratory practicals, the other was used for a pre-session which included all faculty and all laboratory assistants who were involved in the course. Finding a time for this session was not always easy, but we considered it exceptionally important. Since segments of the course changed emphasis and direction frequently form year to year, we could not always be sure that the laboratory assistants would understand what we were doing in any particular week. We therefore met with them and had them do certain selected portions of the laboratory work during that one period.

In assigning student assistants to laboratory time, we found that one hour at a time was better than two or more hours in a block since they became fatigued. Faculty members were assigned to blocks of time when they would be in the laboratory or on call, but they were called upon very little since the faculty member in charge of the course was usually available or present.

Time Cards

These cards (see Figures 1 and 2) were kept in a special wall file which also was used for returning weekly lecture quizzes and weekly laboratory practicals to the students. We emphasized the statement from Open Laboratory 1: "When you come to the laboratory, take your laboratory card from the rack and mark it with the date and time in; and you when leave, mark the time out. It is important that we have these data in planning for work in future years."

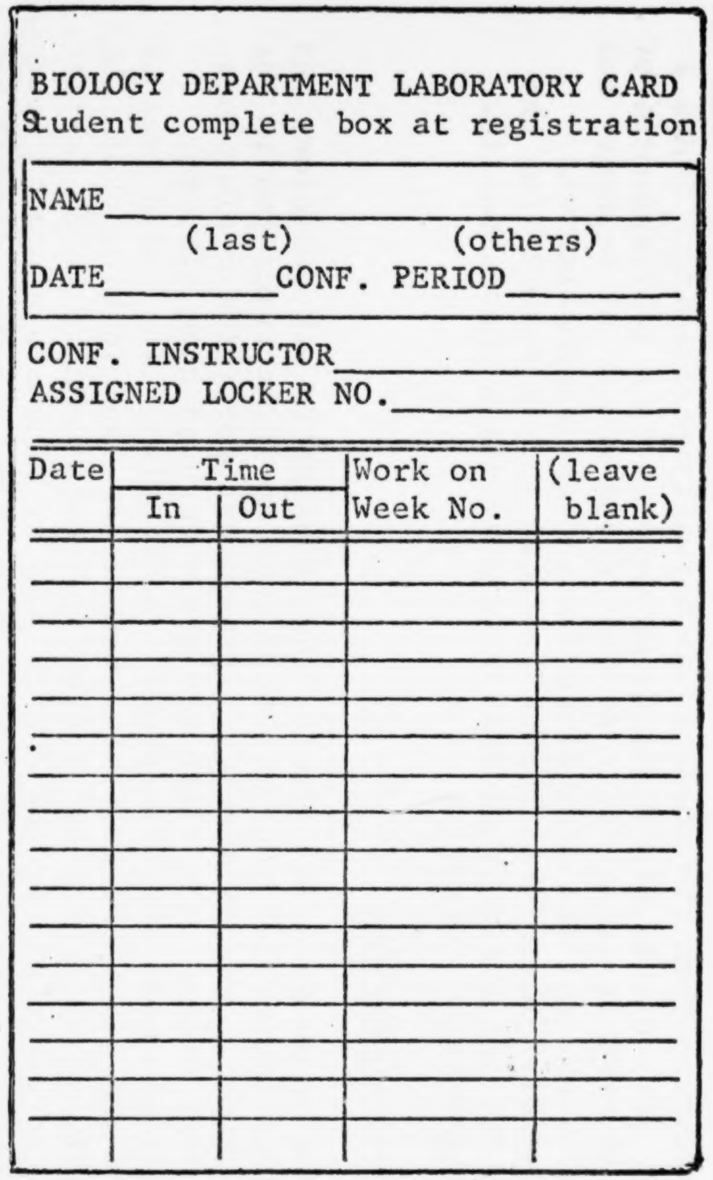


GENERAL BIOLOGY LABORATORY CARD

Name________________________
Lab. drawer ____ Date____________
Conference Section ____________

Date	In	Out	Date	In	Out

Figure 1 time card 1964-66



BIOLOGY DEPARTMENT LABORATORY CARD
Student complete box at registration

NAME________________________
(last) (others)
DATE________CONF. PERIOD________

CONF. INSTRUCTOR____________
ASSIGNED LOCKER NO.____________

Date	Time		Work on Week No.	(leave blank)
	In	Out		

Figure 2 time card 1966-69

Figure 1 was used for two years, figure 2 for the last three years. In figure 2 the vertical columns were continued on the back side of the card. The major reason for changing cards was to include the column entitled "Work on Week No". We found in tallying our results that it was often difficult to tell from a particular date what week's work the student was actually performing. Since a large part of the study was to be devoted to a rearrangement of the laboratory assignments so that there would not be heavy and light weeks for the average student, this information was essential. Therefore, the tally shown in the Results for the first two years are considerably less accurate than those for the last three years.

Conference Periods

We set these up as a time for the students to ask questions and discuss matters of interest that came up in lectures and in the laboratory. No satisfactory arrangement for these conferences was ever found. The first two years we used them also for practical exams and this proved ineffective. The third year we moved the practical tests to another day, and lacking those things to talk about, there was resistance to attendance which was fatal to free discussion. The fourth year we made conference attendance voluntary on the part of the student, and this was somewhat better, because those students who did attend were interested in being there and in discussing the matters brought up in the course. In this way we also could merge some of the conferences that had low attendance. In the last year we abandon the conferences altogether at the end of the fourth week and didnot seem to miss them a bit.

Aside from the three-times-weekly lectures, these were the only parts of the course that were scheduled. All conferences met on the same day and the students could choose the hour when they wanted to participate, limited only by the number of faculty we had available during any given hour during the day. We kept the number between sixteen and twenty in each group, and in a few cases, these groups achieved excellent discussions, but in total the conferences achieved something less than overwhelming success.

Reports

One of the most important aspects of this course was to teach students to write scientific reports. We assigned between five and seven of these per year, depending upon the year (see Appendix 7 for the titles) and this is a heavy burden for the faculty to carry, because each one required a good deal of individual comment. It was here that the conferences achieved their greatest usefulness, since in these sessions the conference instructor could go over the kind of thing that should be reported, how it should be reported, and what he would be looking for when he read the report. Each report was to be returned to the student before the next one was due, as reference for writing the next one, and most students learned to write good reports this way. Each report was designed so that it emphasized one particular phase; that is, either introduction, materials and methods, observations, or interpretations. Of course, certain students did a minimal job on this, but if there is any one thing that students took with them from this course in general, it was the writing of a good report. We believe that this feature of the course did more in showing students how a scientist's mind works than anything we could have done, and we felt that it was well worth the effort.

Practical Exams

Our attitude toward practical examinations evolved over the five year period, but the essential elements were stable enough so that relative scores were comparable from year to year. We thought it was important to give these examinations once a week in order to give the students feedback on their performance in the laboratory and also to insure that the students would keep up in their laboratory work. The latter objective was not always met, but we are certain that it helped. During the first two years we tried several methods, least successful of which was administering them during conference periods.

We decided that since the laboratory was open, we should do the same kind of thing for the practical examinations, administering the test at the student's option, all day on one day a week. Student assistants were assigned to write and set it up in pairs, and the first form of the exam was set up the night before. One of the faculty then checked all the questions that the assistants planned to use for coverage and ambiguity, and to insure that the student actually had had to do the work in the laboratory to do well in the test. For a few weeks each year, most of the items had to be reworked, but since each student assistant was assigned to a practical four or more times during the year, the time that this consumed for the faculty gradually reduced. This was a valuable learning experience for the assistants, and after the initial round, it often saved some time for the faculty.

Practical examinations were given in a separate small room adjoining the laboratory to avoid closing the laboratory on that day; with demonstrations set up in fifteen small carrels. At first we timed the tests, students instructed to move once a minute when the buzzer sounded, so we could admit one student to the room each minute, and one student would come out. An extra laboratory assistant was assigned to that room to admit students, proctor the practical, and distribute and collect answer forms (see Appendix 3). The practical room was available from 7:45 a.m. to 12:40 p.m. and 1:30 p.m. to 4:00 p.m. There were some rush hours during the first of the fall term, but as was the case with the use of the laboratory room, this soon evened out and the room was in use all the time it was open. During the last year of the experiment, at the suggestion of the students in the course, we omitted the timing device, in favor of allowing students to move through the practical at their own rate, the only stipulation we put upon them after a few trials that was each student would keep one empty station between him and the next person ahead. Rather than slowing the proceedings this increased the rate at which students moved through.

The practical was changed completely at least twice during the day, although this could be done in part at more frequent intervals depending upon the schedule of the assistants assigned. Since students were admitted to the test according to the serial number on the answer form, keys were marked "key for numbers 1 to 25" and forms marked accordingly. Questions nearly always had a demonstration, but were not merely naming organisms or parts. We worked to include questions where the student would need to use the information gained in the laboratory in a relatively new situation.

Data Books

Refer to Appendix 7, Open Laboratory 1, Page v for our attitude toward laboratory data books.

We felt strongly that all students, whether majors or non-majors, should keep good records in the laboratory, thereby to learn how a scientist operates. We felt that this objective could not be accomplished by using traditional biology exercises, blanks filled in and turned in for grading. On the other hand, it is important to reinforce the students' learning process by frequent checking. Using the open laboratory, there never was a time during the week when one could collect these data books and have the time to examine them carefully. The best solution was a kind of spot-check in the laboratory, by watching the people as they worked, to make sure they were keeping records properly. Aside from that, there was the end-of-the-term check of the laboratory books, but this was more as a matter of grading, than as a feedback to the students. Thus the Open Laboratory interferes with this one major function, and we have no satisfactory solution for it.

Examinations

A course such as this should not be graded on the basis of only one kind of examination, and we attempted to give every kind possible, in some reasonable mix. The practical exams in the laboratory have been mentioned previously: total recall, answering questions about demonstrations. In addition, in the lecture there was a 20 point multiple choice quiz once each week, which was concerned primarily with facts and theories. The final examination at the end of each term was essay with six to eight large questions. Each faculty member was responsible for writing and marking one or two of these questions.

In the laboratory we gave two individual oral examinations. One took the place of the practicals 11 and 12, and the other took the place of practical 20. (See Appendices 4 and 5 for forms used).

Student Evaluations

Appendix 6 is a facsimile of the student evaluation form used during 1967-68, the next-to-last year of the study. Obviously this form changed from year to year, as various innovations were made in the course. We found such a form extremely helpful in planning for subsequent years. The portion that is of most interest to this report is Part B, Laboratory. These forms were handed out in the last lecture, and students were asked to return and deposit them at the time of the final examination.

RESULTS

Laboratory Directions

The major result of this entire study is included as Appendix 7, the laboratory manuals, Open Laboratory 1 and Open Laboratory 2. Each of these has its own introduction and table of contents, and the reader is referred to those for answers to specific questions and methods. In their original form, these were bound separately by wire, and included a heavy cover for protection in the laboratory. We discovered that there was less confusion for the students if the odd numbered laboratory weeks were printed on colored paper, Open Laboratory 1 in green, Open Laboratory 2 in tan.

Each of the laboratory manuals went through a number of revisions and additions, as we gained in knowledge about what confused students and as we added more illustrations. Only the last-used form is included in this report.

The photographs were taken for the most part with a Polaroid camera and printing was done by photo-offset in the Hamline University Service Department. We kept copies of Open Laboratory 1 and included them as published in this report, but Open Laboratory 2 had to be reprinted from the original plates, and therefore the quality of the printing is not as good as it was originally.

These laboratory manuals, designed to cover twenty weeks, contained most of the information and ideas that we felt a proper course in General Biology should contain. The conscientious student who followed these directions would be well educated in many laboratory procedures and ready to enter advanced courses in biology. That same conscientious student, if he did all the work included, would be overloaded with the course, particularly if he had had no biological background. Our present Core in biology, which separates the non-majors into another course and gives majors six terms covering the topics that are taken up here in 2, was an inevitable outcome.

Number of Students Served

Using the thirty-two-place laboratory described under Methods, the following numbers of students completed each term.

ACADEMIC YEAR	BIOLOGY 11f	BIOLOGY 12w
1964-65	114	94
1965-66	142	112
1966-67	148	119
1967-68	157	117
1968-69	140	101
TOTALS	701	543
MEANS	140	110

The lecture sections were held in a ramped lecture room seating 240, with standard projection equipment. This course normally included 30 to 40% upperclassmen who had no college biology background. Variations in enrollment primarily reflect variations in entering freshmen. The attrition between the fall and winter term reflects the fact that these courses were separable, although 11 was a prerequisite for 12.

Using traditional laboratory sections, these students could probably have been handled in six or seven three-hour sections provided they could be scheduled. Previous experience at Hamline had shown that scheduling students into such laboratories was reasonably easy on Tuesday and Thursday afternoons, which would have given us two full sections, but mornings and other afternoons were not available for blocks of time like this. If we had used scheduled laboratories, we would have needed at least one other laboratory room to handle this number of people, while with open laboratories we could have handled at least 30% more students in one room without crowding.

Comparing this with the previous curriculum at Hamline with three introductory courses, it should be clear that we saved the space previously used for General Zoology and General Botany plus one of the two laboratories previously used for General Biology. From the point of view of space utilization, it would be difficult to find a system better than open laboratory, and at many colleges this is a prime consideration.

Time Spent by Students in the Laboratory

Most of the students were very careful in keeping their time cards, but we found in comparing their time entries in the laboratory data books with their time cards, that some were not so careful. In cases where the two time entries differed seriously, we omitted the data on the card from our statistical study. For all those that we used, we converted each week's time into the number of minutes spent, and then totalled these for each term.

In obtaining this total, however, we were forced to omit two important observations that can only be observed in examination of the raw data and then in discussion with the students themselves.

One observation must be made by examination of the cards themselves, and it is the frequency of attendance: some students would come to the laboratory and perform the entire week's work in a single visit, which sometimes extended for six or seven hours, while others never spent more than one hour at a time in the laboratory, but would come to the laboratory many times per week. Some of this difference in behavior was undoubtedly due to the students' differing class and/or outside work schedules, but in discussing this with many students over the five years it became clear that a lot of the variation was due to the students' own desires. Some said that they could not bear to start something and leave it unfinished, others explained that they could understand the whole week's work better if they took it all at once. Those who attended frequently referred most often to fatigue and impatience if they stayed beyond some stated time. This latter was most apparent when the week's work included extended use of the microscope. The use of the open laboratory allow both kinds of students to utilize the optimum time span. It is interesting to note that those students who attended the laboratory frequently seemed to obtain somewhat higher scores than those who spent long hours at one sitting. A future selective study may confirm this hypothesis.

The other observation can be obtained from examination of the weekly totals, but is lost in the term totals: the extremely variable amount of time spent per week by the same student. It is apparent from this that the students were taking advantage of the opportunity the open laboratory afforded to omit many subjects previously covered and spend much more time on those things they had not done. For example, a student spent a mean of 3.3 hours (1980 minutes) per week in the laboratory, but the raw data show that he spent eleven hours on biological chemistry and only one hour in dissecting a group of invertebrates which he had already dissected in a high school course.

Therefore, in the statistical study that follows, the reader should keep in mind that we are dealing with the total number of minutes per term, and in dealing in this way, one of the major objectives of the open laboratory is lost to view: the great flexibility of the system, that allows the student to assign his own time, make up his own deficiencies at his own discretion, and do it in segments of time that are most effective for him.

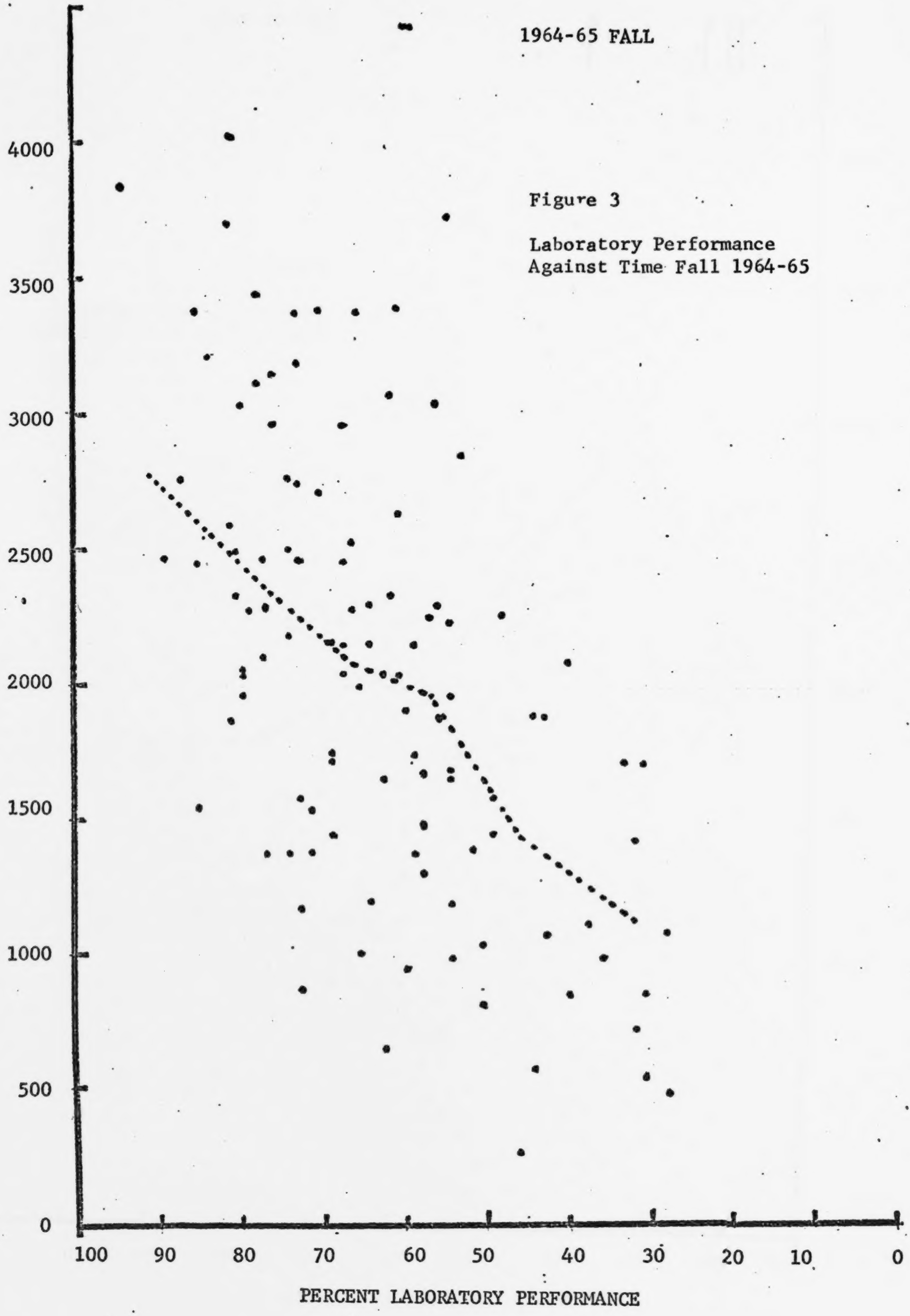


Figure 3

Laboratory Performance
Against Time Fall 1964-65

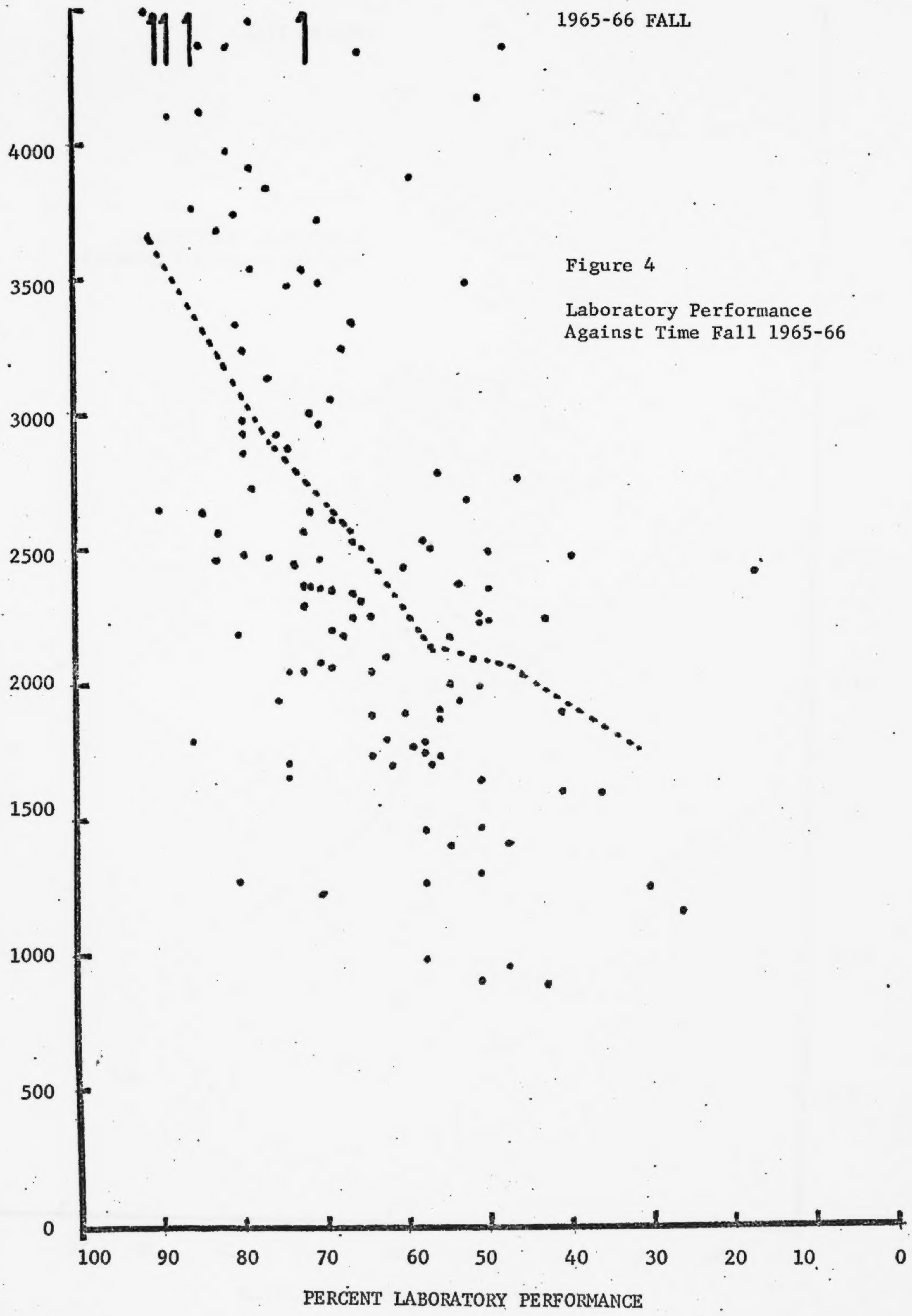


Figure 4

Laboratory Performance Against Time Fall 1965-66

1

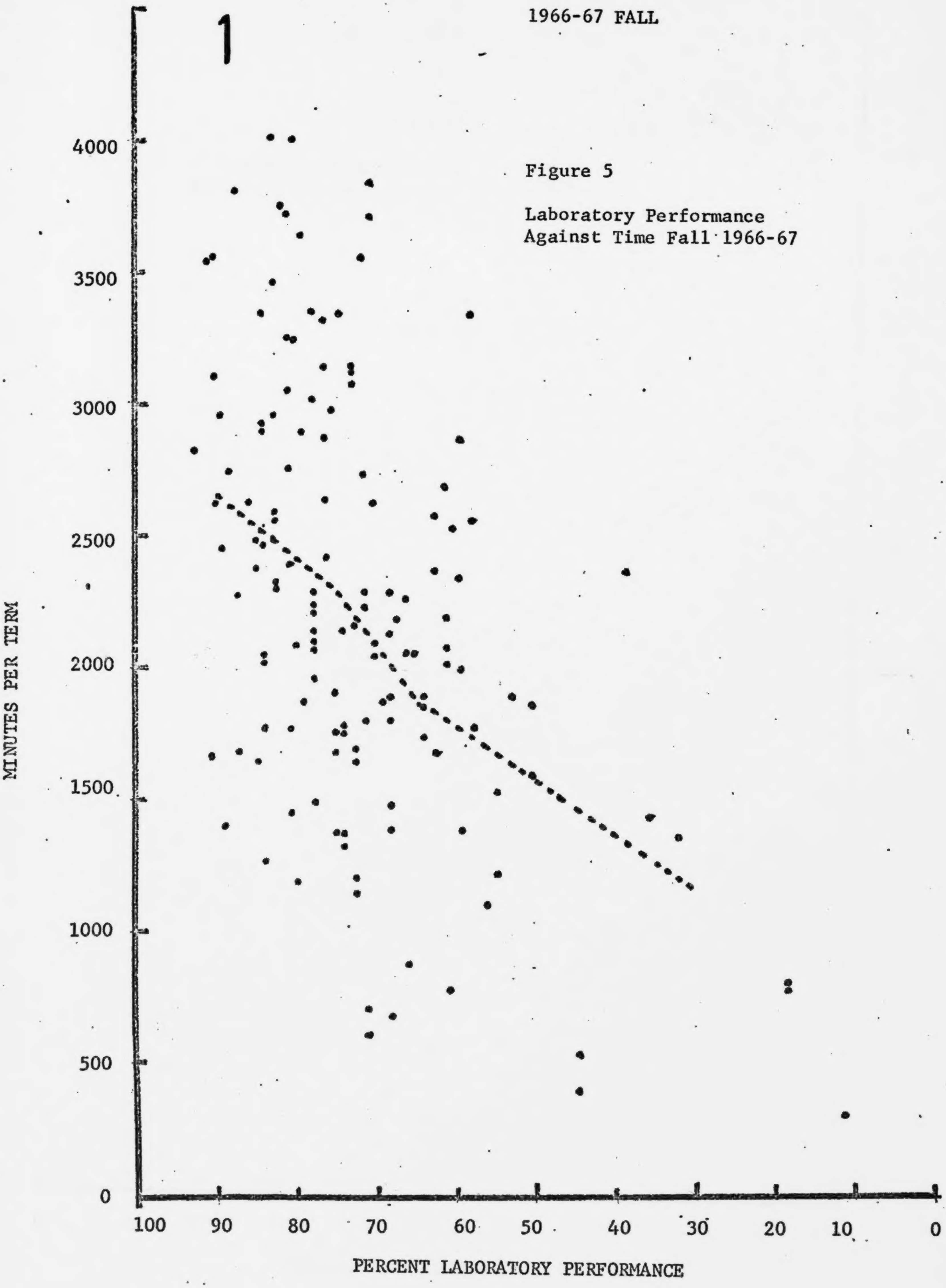


Figure 5

Laboratory Performance
Against Time Fall 1966-67

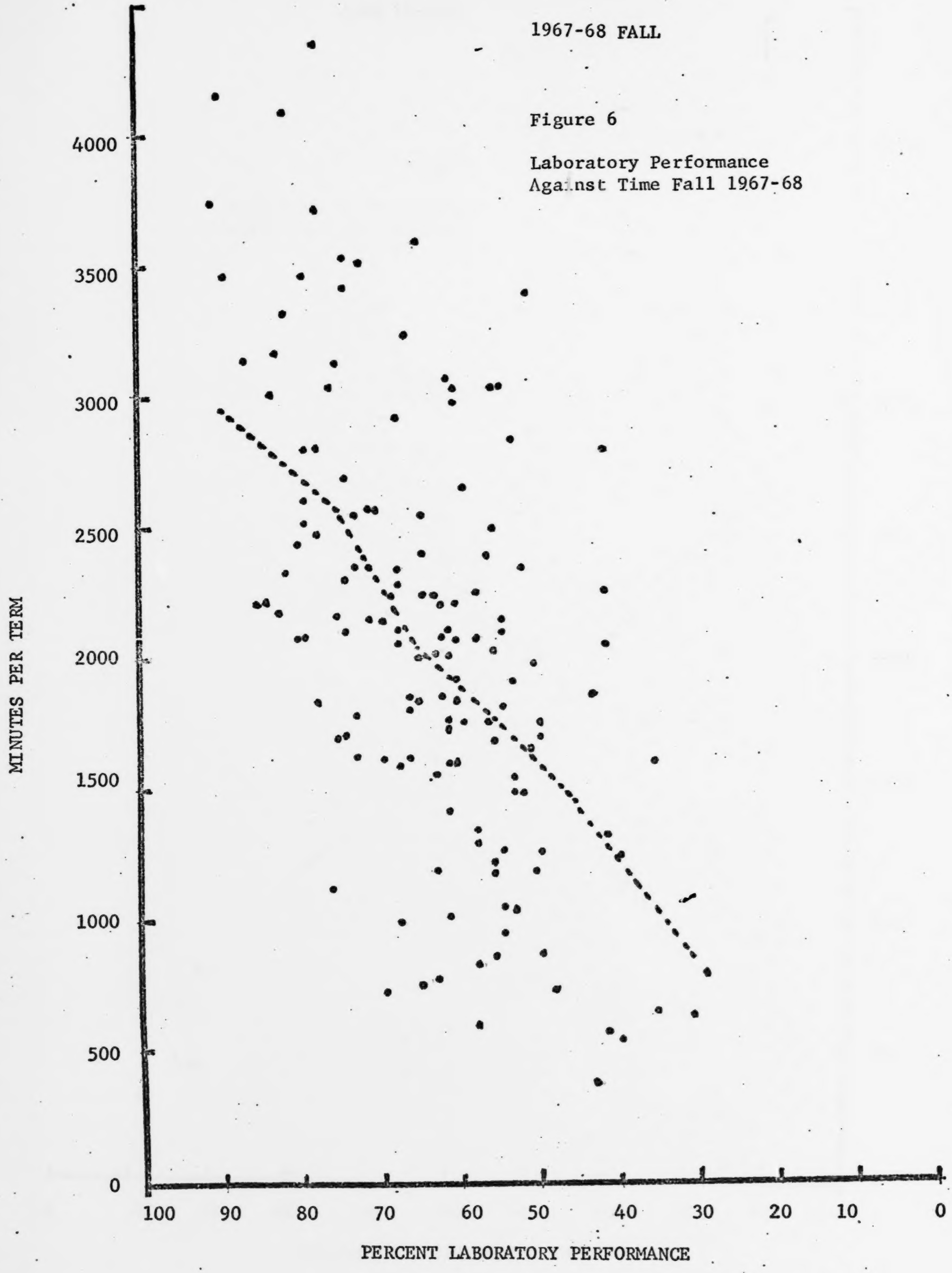


Figure 6

Laboratory Performance Against Time Fall 1967-68

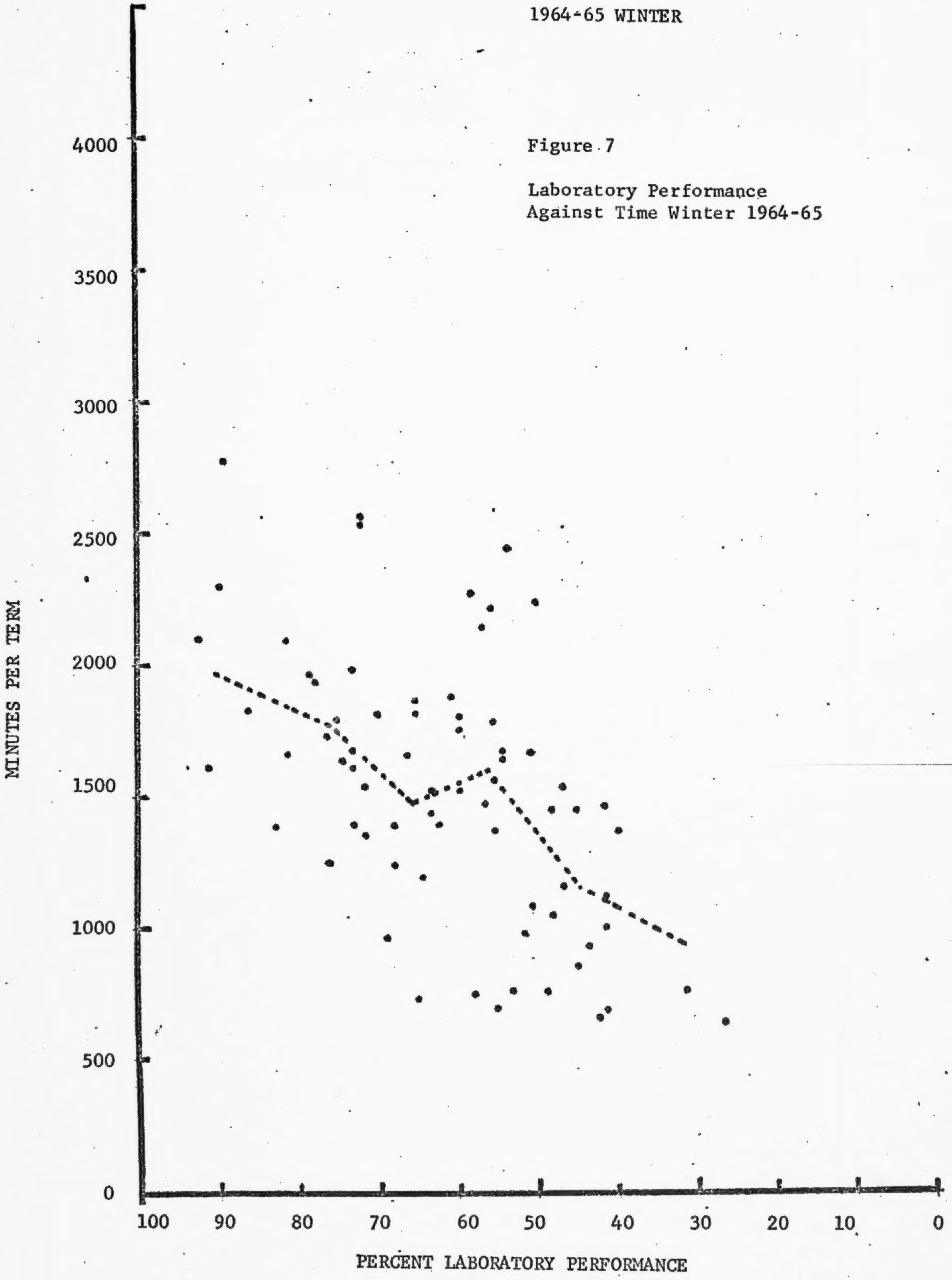


Figure 7

Laboratory Performance
Against Time Winter 1964-65

1965-66 WINTER

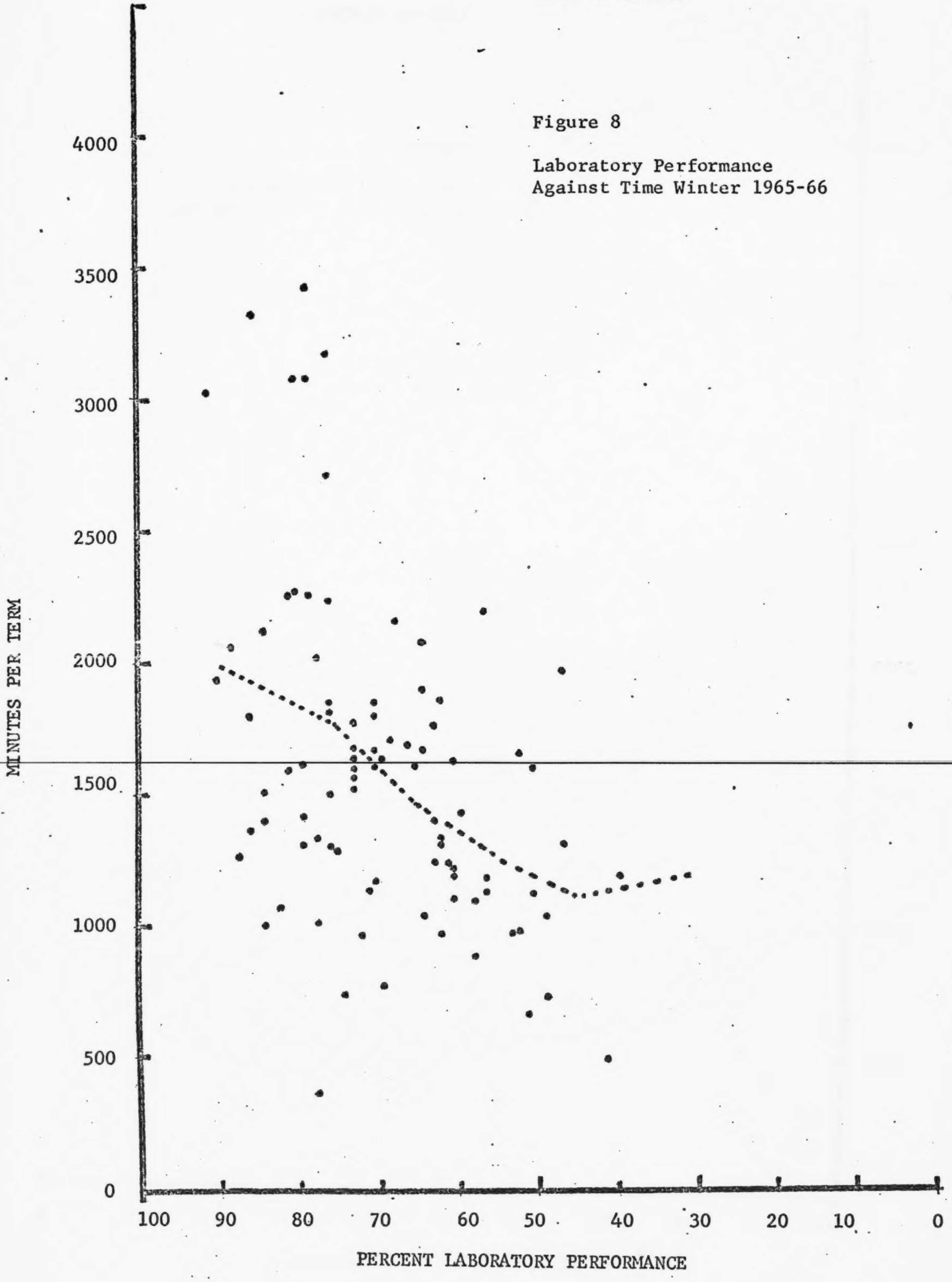


Figure 8

Laboratory Performance
Against Time Winter 1965-66

1966-67 WINTER

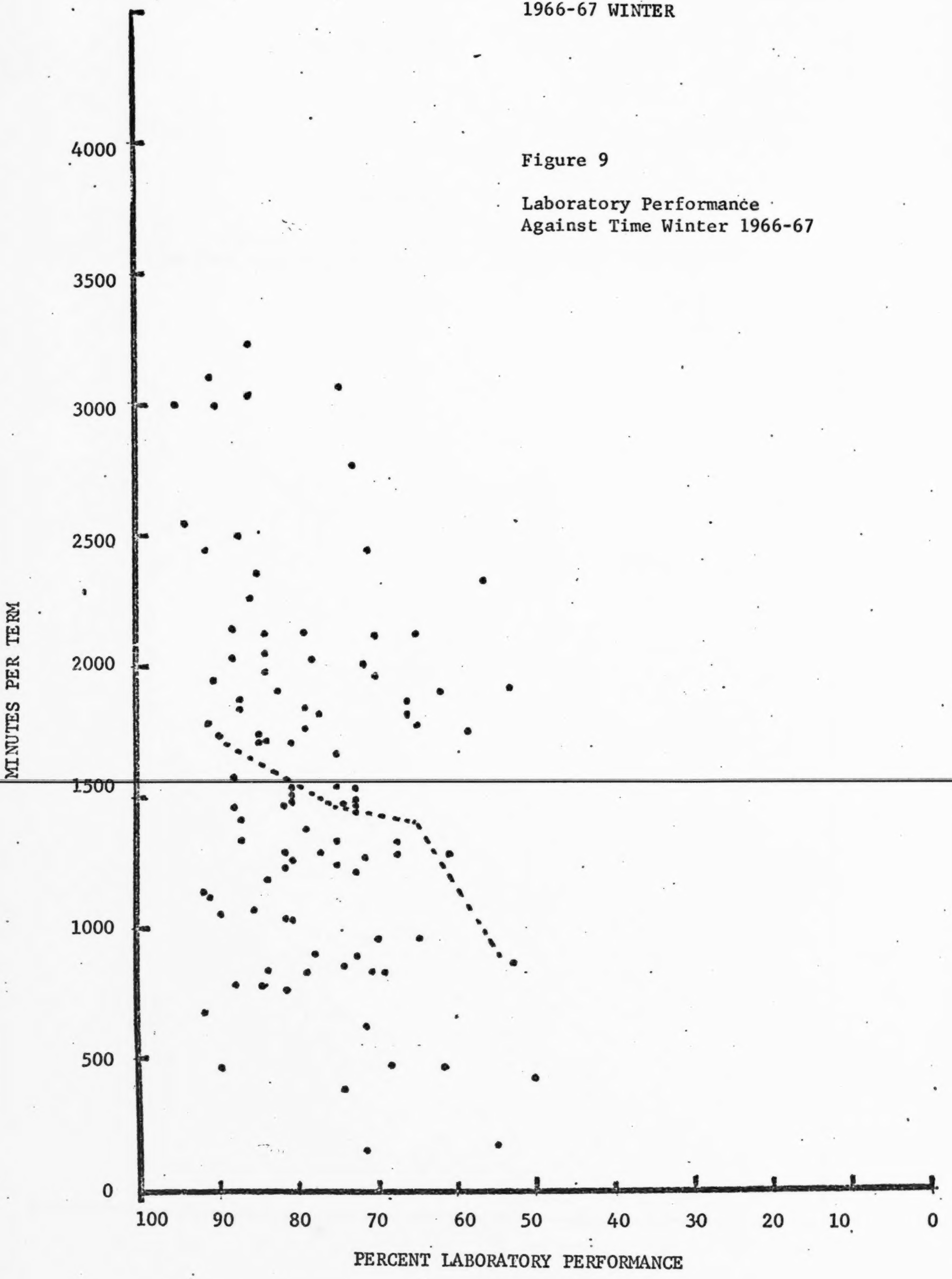


Figure 9

Laboratory Performance Against Time Winter 1966-67

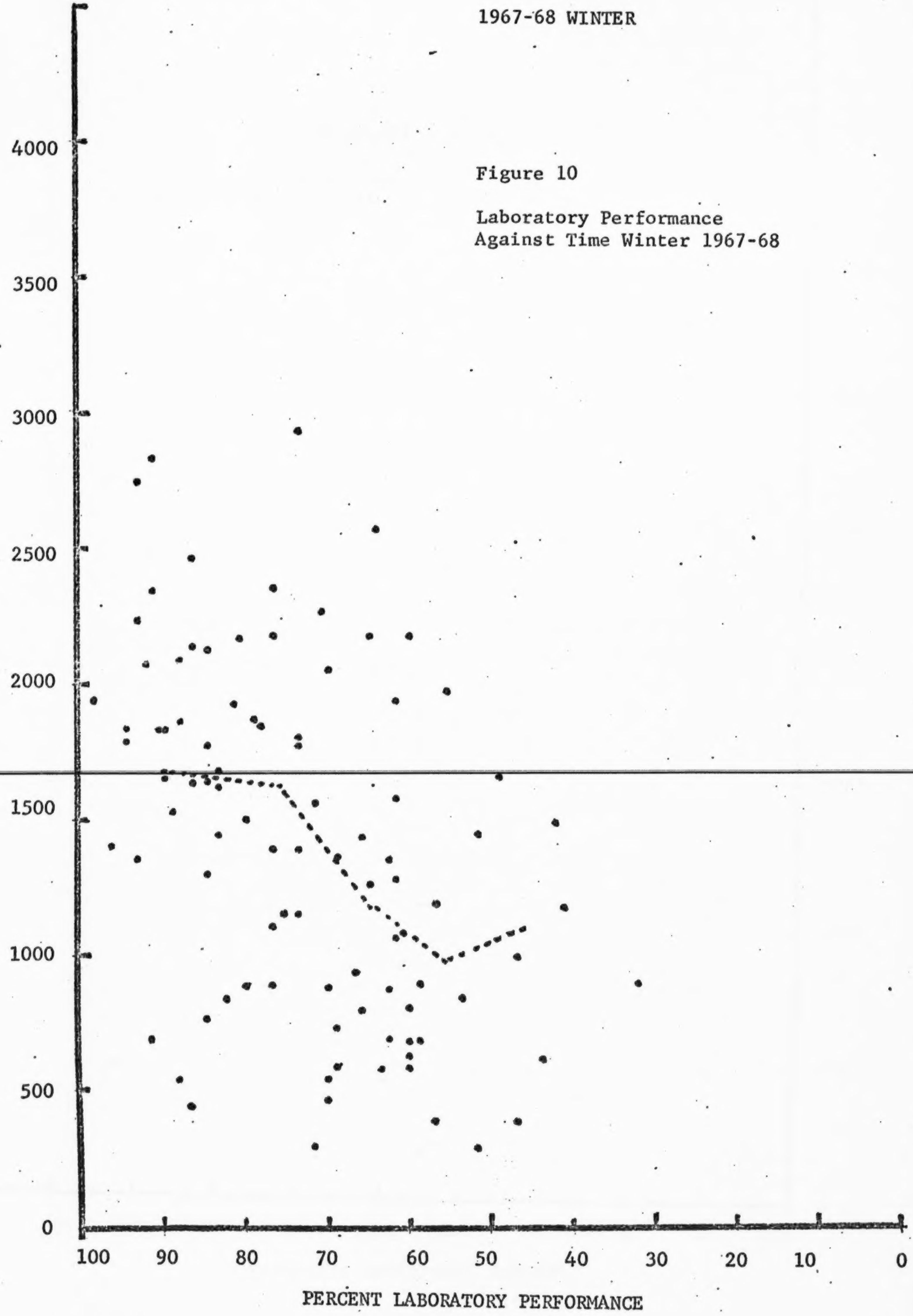


Figure 10

Laboratory Performance
Against Time Winter 1967-68

Table 1

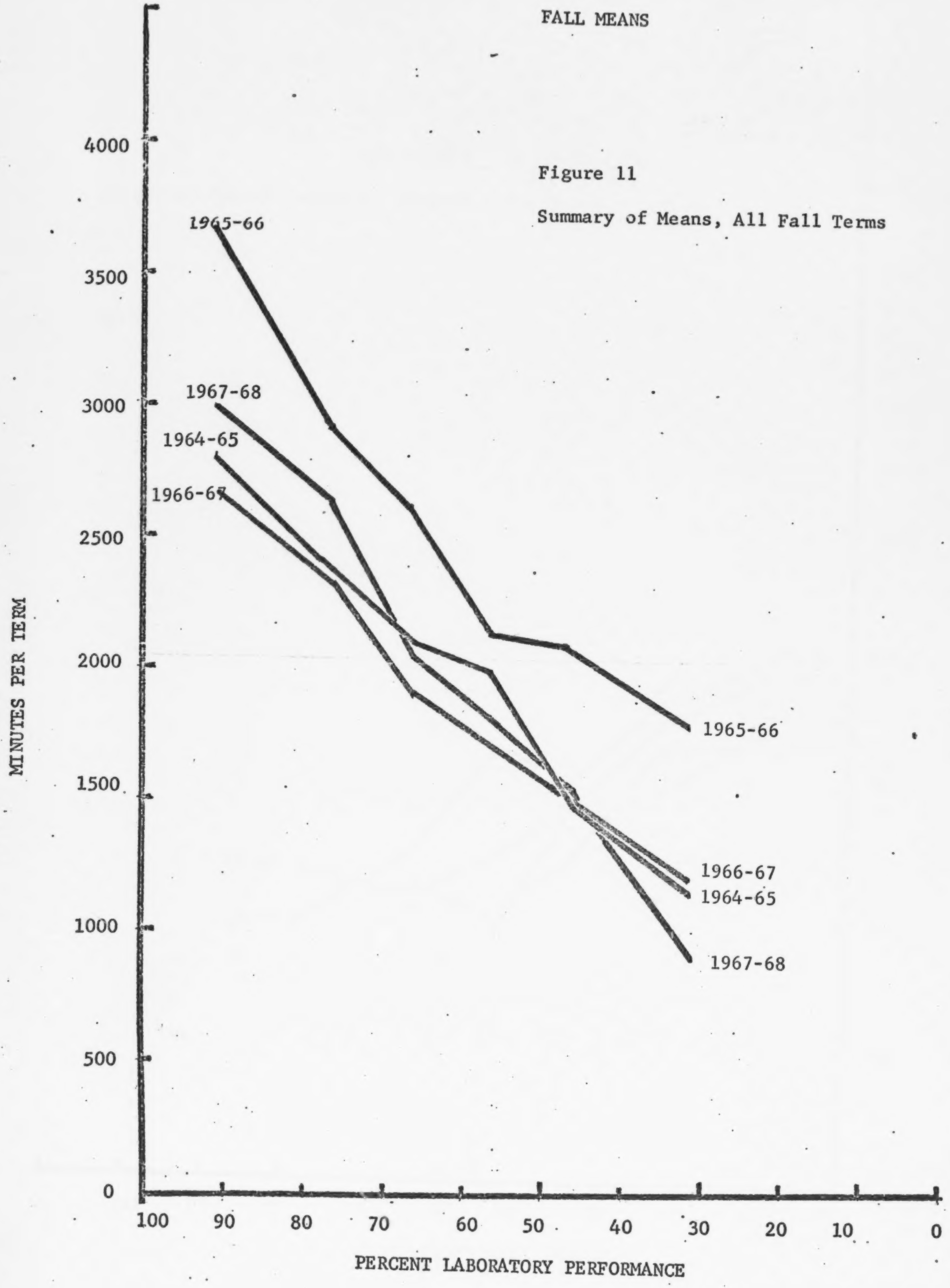
COMPARISON OF LABORATORY PERFORMANCE WITH TIME SPENT IN LABORATORY, 11 Fall Term

PERFORMANCE IN LABORATORY, EXPRESSED AS % OF POSSIBLE POINTS	NUMBER OF MINUTES SPENT IN LABORATORY PER 10-WEEK TERM											
	1964-65			1965-66			1966-67			1967-68		
	MEAN	σ	N	MEAN	σ	N	MEAN	σ	N	MEAN	σ	N
80-100	2794			3672			2666			2995		
		722	10		1092	14		819	45		712	14
70-79	2346			2894			2316			2600		
		767	28		869	34		785	49		778	29
60-69	2094			2555			1867			2032		
		652	22		867	27		514	26		631	45
50-59	1971			2101			1938			1793		
		725	24		623	25		640	13		725	37
40-49	1467			2006			Unreliable			1456		
		843	11		959	18					703	13
Under 39	1117			1755			1150			853		
		489	12		491	7		649	6		387	5

Table 2

COMPARISON OF LABORATORY PERFORMANCE WITH TIME SPENT IN LABORATORY ,
12 Winter Term

PERFORMANCE IN LABORATORY, EXPRESSED AS % OF POSSIBLE POINTS	NUMBER OF MINUTES SPENT IN LABORATORY PER 10-WEEK TERM 1964-65			1965-66			1966-67			1967-68		
	MEAN	σ	N	MEAN	σ	N	MEAN	σ	N	MEAN	σ	N
80-100	1987			1972			1715			1670		
		420	8		685	15		683	49		552	32
70-79	1796			1717			1488			1607		
		387	14		633	31		641	33		614	17
60-69	1457			1473			1407			1178		
		351	13		363	22		595	15		577	22
50-59	1615			1229			807			954		
		548	20		409	11		611	4		563	13
40-49	1161			1067			Unreliable			1094		
		421	14		515	5					417	6
Under 39	906			Unreliable								
		324	3									



FALL MEANS

Figure 11

Summary of Means, All Fall Terms

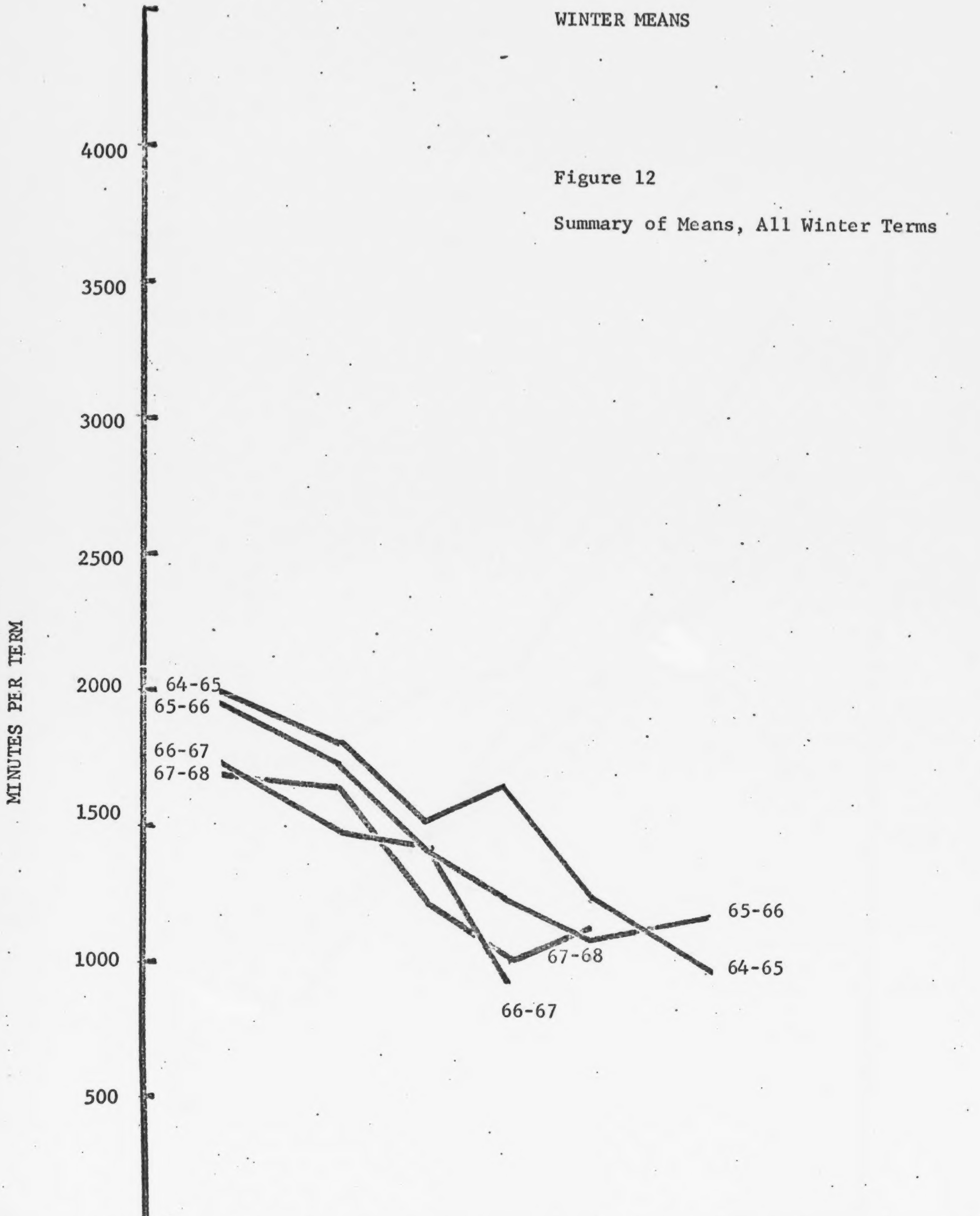


Figure 12

Summary of Means, All Winter Terms

Comparison, Laboratory Performance with Time Spent in Laboratory

The student's final letter grade in this course took into account not only the laboratory work, but also the lecture quizzes and the final examination. It was thought therefore that it would be unfair to the data from the laboratory to compare its time against the final grade in the course. Instead for each term we produced a figure for each student called "laboratory performance". We took a total of points that could be earned on laboratory practicals, reports, and laboratory data books, then expressed each student's laboratory performance as a percent figure of the total possible.

Figures 3 through 10 show this laboratory performance plotted against the time spent in the laboratory for each student for which we had reliable data. Figures 3 through 6 consider only the fall term, while 7 through 10 show only the spring term. Data from 1968-69 are not included since the time cards were stolen from the wall racks on the last day of the fall term of that year.

The dotted lines on each figure are graphic representations of the information in Tables 1 and 2, with the means plotted respectively at 90, 75, 65, 55, 45 and 30. We found that the number of students (N) who achieved between 80 and 100% was so small that we combined them, and also combined those under 39%. We produced σ through the use of a program on the Wang Calculator.

It is apparent that the same laboratory performance required highly variable time in the laboratory from different students, but taken as a group there are correlations between high scores and high number of minutes, and between low scores and low number of minutes. It is doubtful, with N so small and σ so large, if the mean value has any particular significance, but assuming for a moment that it does, then one may observe the same general kind of slope for the line connecting the means in all the figures. Figures 11 and 12 summarize these connecting lines, for comparison.

It is a widespread assumption that students who spend more time working or studying will achieve higher scores than those who do not, and these data seem to bear out this assumption (if the mean scores have any significance). It would be risky, however, to ascribe cause-and-effect relationships with the data open to such question.

Student Evaluations

We made a great deal of use of these forms, primarily in making modifications for future years, and consequently, the form changed from year to year. Therefore, though similar, they cannot be summarized on a total basis. Some data of importance to this study are given here for the typical year, 1967-68. Numbers of the questions are those that appear in Appendix 6.

11. Suggestions have been made that the Biology Department should institute a course for non-science majors (something like "Fundamental Concepts of Biology"), and confine 11 and 12 to biology for science majors. There are several problems involved in this. Insofar as you can envision the problems, do you favor this idea? Yes 53% No 37% Indifferent 10%

13. Do you think that the text, Weisz, The Science of Biology is too difficult? Yes 33% No 52% Indifferent 15%

18. The alternative to Open Laboratories is two two-hour laboratory periods scheduled per week, with the laboratory continuously occupied. Do you prefer Open Laboratory?
Yes 78% No 15% Indifferent 7%

19. Laboratory practicals were given weekly this year. Do you think it would be better to have them twice as long, but given every other week?
Yes 8% No 88% Indifferent 4%

20. Do you like this year's system of having students take the laboratory practical at any time that they are ready, unscheduled? Yes 94% No 5% Indifferent 1%

21. Do you think there should be some non-practical questions included in the lab practicals?
Yes 25% No 66% Indifferent 9%

22. The photographs in the laboratory manual were new last year. Their purpose is to help the student find things and demonstrate procedures. Do you think they achieve their purpose? Yes 74% No 16%
"Usually" or "sometimes" 10%

In Question 24 we rated the responses quantitatively, and then ranked the 20 laboratory weeks from Greatest Value to Least Value under the heading Student Opinion of Value.

11 Arteries
10 Vertebrate Structure
12 Veins of a Bullfrog
17 Metazoan Embryology
4 Animal-like Protista
20 Genetics
8 Annelida and Arthropoda
16 Mitosis and Gametogenesis
14 Gross Morphology
5 Plant-like Protista
9 Mollusca, Echino
3 Monera
1 Materials and Methods
7 Platyhel and Nematoda
6 Porifera and Cnidaria
19 Angiospermae
2 Biological Chemistry
13 Mesodermal Derivatives
18 Non-flowering Metaphytes
15 Epithelium Histology

After checking Appendix 7 the reader may want to ascribe his own reasons for this group opinion on the part of the students. Of course, it is not always good pedagogy to modify directions on the basis of student opinion, but one can see how interesting a tabulation like this would be to the person writing the directions for the following year.

CONCLUSIONS

Cancellation of General Biology

The termination of this study was also the end of General Biology as such at Hamline University, and the things we learned during the five-year study were of considerable influence in making the decision to cancel. Although abandonment of the method of open laboratory accompanies the cancellation of the course, it should be emphasized that what we rejected was the idea of General Biology, rather than that of the open laboratory itself, and we rejected General Biology because the open laboratory worked well enough to point out the intrinsic problems in such a course. The new curriculum containing the courses that will now take the place of General Biology was under discussion during all of 1967-68, making the basic particular course decisions consumed all of 1968-69, details included within the particular courses were decided during the summer of 1969, and most of the courses in the resultant core and non-major courses are started or will be started during 1969-70.

If this final report of the study had been submitted any sooner than the present, these conclusions could not have been as complete as they are, and the section on Recommendations could not have been included at all. We are grateful to those who perceived our dilemma and unofficially agreed that extension of time was justified.

Sequence of Conclusions

Obviously a teaching method cannot operate in a vacuum, but must be used as a part of some course, and since our open laboratory study was tied to General Biology for its content, the operation of the method was inevitably some function of that course. Although therefore it is impossible to adhere to the sequence rigidly, the conclusions follow this pattern: first an evaluation of the method of open laboratory as we used it; then an assessment of the entire course in General Biology as we taught it; and finally the concept of a course in General Biology in whatever form it is taught. In the Recommendations will be found an outline and rationale of our new core curriculum, which is what we have recommended to ourselves and which we herein recommend to others as a solution to the General Biology problem.

Laboratory Manuals

The study was primarily concerned with evaluation of open laboratory procedures, and the bulk of that evaluation was written into the laboratory manuals, Open Laboratory 1 and

Open Laboratory 2, included as Appendix 7 of this report. This evaluation was a perpetual process during the five years, and the author is reasonably satisfied with the results as published here. As they now stand, they do what he feels laboratory directions in General Biology should do; clearly explain the procedures and techniques necessary for good understanding of the subject. Obviously there are some procedures omitted, that would have been included with acquisition of new equipment by the department if the course had continued. Also as with any published gelation of directions in transition, there is some need for improvement in wording, accuracy and clarity of directions, but these improvements will not now be made to these volumes.

In 1969-70 we ceased to use the present published forms of the directions, but the work on them was not wasted since we are now finding that many of those weeks' work are directly transferable to the core courses and/or non-major course and are exceptionally useful. The studies made on record-keeping and report-writing were also most helpful, and these procedures have moved intact to courses in the core. Thus though the directions in toto were too time-consuming, they contained many parts that will continue to be useful.

Faculty Reaction to the Method

Any new approach or curriculum has the potential of receiving approval and good results during its time of initiation, simply by virtue of the fact that it is new. The biology faculty at first was concerned over the single course as prerequisite for all other courses in the department, but none expressed anything but relief in losing the full responsibility for separate laboratory courses. This approval continued for the first two years, then began to decrease as changes in the faculty took place and other factors became more important.

Student Reaction to the Method

Student approval of open laboratory was strong at first and continued at 78-92% annually in the student evaluations, even in the last year when the students knew this was the last year that this approach would be used. In interviewing students about their stand, the strongest reason was important and widely held: the open laboratory treats students as adults, in forcing upon them many decisions as to time spent and content covered, and they liked to have the chance to make their own mature decisions about when to come to the laboratory and how long to stay.

Efficient Use of Facilities

Efficient use of the laboratory may to some be enough observation to force the conclusion that such a system must be used, regardless of its detrimental features. This has been demonstrated clearly in the past few years by the many departments that have adopted Audio-Tutorial laboratories in which great numbers of students can be "handled" in a small space, using taped mini-lectures, projected demonstrations and already-completed experiments, in which the student moves from station to station answering questions, filling blanks and drawing conclusions from evidence presented. We feel that drawing conclusions is the easiest part of science, and that we want to present laboratories in which the important part of science is forced on the student, at least sometimes: the asking of pertinent questions of the material, and the setting up of experiments to answer the questions they ask.

Our department does not need to make the saving of space a prime consideration, but any department that does, and is considering audio-tutorial laboratories as a solution, should compare advantages of open laboratory methods with that system first. Open laboratory has the space-saving virtues of that system, without its initial expense, upkeep, mechanical problems and rote-learning disadvantages. Neither faculty nor students are tied to tapes, films or demonstrations.

Time Saved By Students

Saving time for the student is open to varying interpretations, depending primarily upon the background he brings to the course. It is obvious that for the individual student the open laboratory can save time, or can take more time than a scheduled period would, depending on the time he needs and/or spends in doing his work. The student with some kind of biological background saved the time a traditionally scheduled system would have spent on things he already knew, and he could use that time on biological chemistry. This allowed us to teach the course assuming some chemistry background on the part of all students, and this freedom made it possible to cover up-to-date subjects we would otherwise have had to skip.

The greatest majority of the students in our course had no chemistry background, and therefore the above fit most of those in our course. However, the student who had a background in chemistry did not save time but lost it, since he was held up for weeks in our course because the majority of the students needed to have biological chemistry explained from zero. Often those students with chemistry were those heading toward biology majors, and thus unwittingly we bored and discouraged those very students who we wanted to attract and stimulate. An important conclusion, therefore, is that, if an up-to-date course in biology is to be presented, it cannot be truly efficient when some of the students have an extensive background in chemistry and others have none at all. Sectioning, if it is to be done, should be on the basis of chemical rather than biological background.

With the open laboratory we provided time-saving and flexibility during the time when BSCS ideas were moving into the high schools, but these ideas have now become widespread enough so there is now more uniformity in biological background among entering college students than there was five years ago. During the transition period, however, our presentation of both traditional and newer approaches to biology in our course allowed all students to arrive at a common theoretical basis for biology, and the flexibility allowed them to take advantage of whatever previous biological background they had.

Records and Reports

As we taught our course, there was strong emphasis on good record keeping in the laboratory and the writing of good reports. Although the open laboratory itself prevented our frequent examination of the laboratory data books, we would urge those who adopt open laboratory to maintain this emphasis and find a solution to the feedback problem. The open laboratory provides the structure for the students to truly experiment (i.e., ask appropriate questions, plan an experiment to answer them) as well as to draw conclusions, as a basis for good reports, and this writer feels that no other plan does this as well. Students in our core courses will also be writing reports, and we will use the experience we have gained from this study in teaching students how to write them, but the open laboratory framework provides the ideal timing for students to do the work.

One interesting incidental observation should be included here: In looking for numerical data that have the closest correlation to final grades in the course, we found that the scores earned in reports have the closest fit; better than time spent in the laboratory, better than lecture or laboratory quizzes, better than laboratory performance. This is true even though the points granted on the reports are not enough to have much bearing on the final grade. Perhaps the kind of mind that can produce a good report is, after all, the kind of mind that is found in a good scientist.

Frequent Tests

Both the faculty and the majority of the students involved felt that it was important to test learning frequently, preferably a weekly test both in the lecture-theory and in the laboratory to give rapid feedback to the students in this course where grading authority is diffused. In invited lectures (see Faculty Lectures below) each faculty member was in sole charge of two lecture quizzes per term, which gave him a voice in the final grade. Laboratory practicals were weekly and under the control of the instructor in charge of the course. The student therefore had twenty tests each term, which does not seem excessive. A part of the final examination at the end of each term was written and graded by each individual faculty member.

Student Laboratory Behavior

Laboratory disorder is promoted by the open laboratory, since students do not have a space assigned but only a locker. We avoided some disorder by checking locker materials out to the students and making them financially responsible for losses, but this did not prevent some of them from leaving litter when they left, since they had occupied only an anonymous work area while in the room. An important faculty objection to the open laboratory stems from this problem: one of the most important things to learn in laboratory is proper behavior, responsibility for cleanliness and order, and students were not trained in that laboratory procedure in this course. They kept good records, they wrote good reports, they did fine independent projects, but they occasionally did the work leading to those ends among general confusion.

Perhaps the loss of this training is somewhat balanced by students learning to perform their work quickly without wasting motions, given the open laboratory situation in which this behavior is rewarded. This tentative conclusion is borne out to some extent by comparing Figures 3 through 6 with Figures 7 through 10, which show respectively the laboratory time in the fall term with that in the winter term. The time spent during the second term overall is considerably less than in the first. This led to the hypothesis that a study of the week-by-week times would show a steady decline in time spent by each student during the two terms. A preliminary study of the weekly totals indicates that this is a strong possibility, but time limitations in an already-overdue final report prevent the completion for inclusion herein.

Faculty Responsibility

A minor faculty objection to open laboratory was too many hours' responsibility for the laboratory itself. Each one had five or six hours per week when he was on call by the assistant. The laboratory was open for thirty-nine periods per week, and needed a faculty member on call during twenty-eight of them (exceptions: second hour, Tuesdays and Thursdays; seventh hour, daily; first through fourth hour, Saturdays). The instructor in charge of the course assigned himself about ten of these periods, and the remainder were divided among the other three faculty. If we had had assigned laboratory sections of three or four hours each, these students could have been served in seven sections which would have taken twenty-one or twenty-eight periods, and would have saved up to seven periods of faculty assignment. This criticism loses much of its validity on closer examination, however, since at Hamline our scheduled laboratory periods require the nearly continuous presence of the faculty member, while in the open laboratory the instructor was only on call, not necessarily present, and the student assistant was the staff during most of the time. This, incidentally, made faculty load almost impossible to compute for the biology faculty, since the University administration had no load formula for the "on-call" situation.

Faculty Reaction to Course Content

The biology faculty strongly approved of one phase of our course in General Biology: that we concentrated on general principles, and were concerned with biology and not with the taxonomic subdivisions. This was a distinct change from the previous major curriculum at Hamline, which was oriented around beginning courses in zoology and botany, plus a non-major course that attempted to cover Biology. We could see from the beginning that such orientation would not be viable in present days when biological facts are increasing logarithmically and the generalizations in all the biological sciences are coming ever-closer together. One must find and present the generalizations about all living things, illustrating them with the most appropriate forms, without restriction to plants, animals or bacteria. In our 1964 course we moved intentionally toward emphasis on biological principles and generally-applicable laboratory procedures, and gained a new departmental attitude which we would not have gained without it.

The positive response of the biology faculty to this new direction was contrary to its response to the open laboratory which started strong and became weaker; this approval began in the second year and increased rather than decreased during the five years. It has now culminated in our orienting our entire new biology core curriculum around these ideas. Each course in the core considers all kinds of living things, and fits them into the principles, rather than into the taxonomy.

Faculty Lectures

We were determined to present the most appropriate faculty member for each topic in the lectures, and tried several methods to do so. Student reaction in the annual Course Evaluation was helpful in determining our effectiveness (see Appendix 6, item 2 for wording of the 1967-68 question). In what we called "team teaching", the emphasis was on the logical topic sequence. We divided each 70-minute period into two parts, and assigned fifty topics per term to these time segments. Each faculty member chose those topics he wanted to discuss, and as a result sometimes one member might lecture for a full week (five topics) and at other times he might have no topic or only one of the five. Weekly lecture quizzes were made up jointly by the lecturers who spoke during the previous week. This seemed in prospect to be a better idea than it turned out to be, because faculty members usually were unable to develop ideas sequentially with this plan and students felt this discontinuity. This negative aspect might have been avoided with longer use.

What we called "invited lectures" attempted to bring more continuity. The instructor in charge was scheduled for the first two weeks to present an introduction to the term's topics and organization, then each faculty member was given a full two-week period in which he developed a complete set of ideas, prearranged by the instructor in charge. This was probably the best solution we found. It allowed each member to have four weeks (two each term) to form a logical foundation for his own later courses in the department.

Faculty Interrelationships

To be able to place all beginning students in one course, in charge of one instructor, and thereby release the other faculty from sole responsibility for separate beginning courses, it is necessary that the other instructors involved assume a subordinate role. Great care must be taken by the instructor in charge to insure that all policy decisions either be made by the group as a whole, or that his colleagues be kept continuously informed, if the department intends to maintain such a course beyond its initial trial period. It is here advised that the group involved meet on a regular basis, by themselves without student assistants, to discuss the procedures and policies of the course.

A Course in General Biology?

The objectives of a course in General Biology are usually considered to be excellent. A course that presents all the truly biological principles, properly illustrated in the laboratory from among all living things, would surely be of great value to the liberal education of all undergraduates, and particularly to the education of biology majors.

Too often, when separate courses are presented for non-science and science majors, the course for the liberal arts students contains the breadth, relevance and overarching principles that we should be teaching to our majors, while the course for majors concentrates on details of theory and laboratory technique to the detriment of biological relevance. In our anxiety to get these majors ready for graduate school, we forget that the undergraduate experience is the one place where these potential specialists can form the value judgments that will allow their remaining lifework to make sense to them. A genuine course in General Biology, which carries out the above aims, should be the most valuable course he takes.

But these objectives must raise some questions in the minds of earnest biologists who have been attempting to achieve them with mixed success ever since such courses became fashionable several decades ago. These questions all center on one major one, every year made more urgent by the explosion in biological knowledge: Can it be done?

The answer to this could be affirmative, in which case a number of subsidiary questions arise. Will the same course serve equally well for the non-science student and the biology major? Are their goals similar enough? Are their backgrounds similar enough?

Our answer, however, at the present time is in the negative, based primarily on a matter of timing. For the non-science student there are so many basic principles from all of *science* that need to be learned first, before present-day biology can be approached clearly, that there is not enough time remaining in two terms to cover more than a few of the most important *biological* principles. The biology major, on the other hand, has turned his education toward biology because of an interest in science, and therefore has those scientific principles *already* in his background. What he needs from General Biology is a biological basis for his later specialized courses. True, he does need to study biological principles, but needs to consider them at the level when he has the biological facts and theories thoroughly learned.

Principles need a great deal of example and illustration to be memorable, and it seems to us that two terms of General Biology do not provide enough time for this expansion. Principles started in General Biology, planned to lay a groundwork for later departmental courses, are forgotten by at least some students before the later courses come. We feel this is due to the lack of depth at the outset, and the lack of depth is due to a lack of time.

The primary reason that we abandoned General Biology at Hamline is that we, as a faculty, came to believe that such a course tries to do too much, and it cannot do what it professes to do in two terms without overload or omission of vital portions. By providing the laboratory time in which to do what should be done, we showed with this study both that such a course places a burden upon the time of students who do not plan to major in biology, and that the material learned is too little for those who do plan to major in biology.

Our solution to the problem of too much work for the non-major students is to teach a course that would not be considered by biologists as General Biology or even a survey of biological principles. This course should be based on the value judgments about biological subjects that the educated citizen will be forced to make in the future; it should ask the questions that will come up in their future lives, give enough of the biological principles to make sense of the questions, and enough facts to begin making answers. The laboratory should be brief but illustrative of the questions. This kind of course is not new to biology, but is new to us, and we are looking forward to developing it.

Our solution to the problem of superficiality for major students is to begin with a background in chemistry, and take students for a term each, going into as much depth as possible in those courses. The five core courses will then be summed up in a sixth core course, which will do in fact what General Biology attempts to do -- present general principles, but develop these general principles from the more specialized courses, not lead to them.

RECOMMENDATIONS

The recommendation that we have made to ourselves, and are putting into operation this year, is to adopt a core of courses for majors, based on chemistry, and a separate sequence for non-majors which will cover only a few topics. We present the following set of recommendations extracted from biology faculty action taken in 1968-69 for those who wish to see what has grown out of a long study that appears at first glance to be a failure. These recommendations are based on a year's hard work by a committee of four faculty and eight biology students.

Rationale

The adoption of a new Biology Core Curriculum at Hamline University in 1969 was an innovation designed to modify and improve the way in which majors study biology. In a traditional curriculum, a year of General Biology is the usual prerequisite for all other biology courses. Such a survey usually attempts to introduce all the major biological generalizations, cover some of them superficially, and then the students return to them in later specialized courses. We have decided to drop this method in our Core, because the rapid increase in numbers and kinds of generalizations has forced the Survey to become either too superficial or too time-consuming. Instead we take up the biological principles in five courses a few at a time in depth, and then synthesize biological knowledge in the last Core course.

The intention, design, and prerequisites for Core courses are different from those for required courses in a traditional curriculum.

A Three-Part Program

Adoption of a Core divides the biology curriculum into three parts. Course descriptions are given here only for the non-major sequence and the core courses.

COURSES FOR NON-MAJORS

13 Biology of Organisms. Living things: their diversity, nutrition, growth, reproduction, significance in the balance of nature, and importance to mankind.

14 Biology of Cells. Living cells: the pattern in diversity: their origin, metabolism, replication, genetic continuity and maintenance of function in changing environments.

CORE COURSES FOR MAJORS

15 Cell Structure and Function. First course in the Biology Core Curriculum. The interactions of morphology and physiology at the level of the cell, organized around the phenomena observed in living cells in the laboratory.

16 Organismic Form and Function. Second course in the Biology Core Curriculum. The interactions of morphology and physiology at the level of the organism, organized around the phenomena observed in living plants and animals in the laboratory. Prerequisite: 15.

27 Genetics. Third course in the Biology Core Curriculum. The continuity of cells, the maturation of gametes, Mendelian and non-Mendelian genetics, the form of individuals resulting from crosses, the stability and change within populations. Laboratory gives practical experience in crossing fruit flies and molds, and observing genetic ratios in plants. Prerequisite: 14 or 16.

28 Ecology. Fourth course in the Biology Core Curriculum. Comparative studies of structure and function in various types of ecosystems and factors causing space-time variations in structure and function of ecosystems. Prerequisite: 16 and 27.

29 Developmental Biology. Fifth course in the Biology Core Curriculum. Reproduction, development and evolution at the levels of cells and organisms, organized around the phenomena observed in developing plants and animals in the laboratory. Prerequisite: 16 and 27.

80 Seminar and Teaching. Sixth course in the Biology Core Curriculum. Two seminars per week which emphasize synthesis and application of biological principles. In addition, each student is in charge of one conference and its corresponding laboratory section in the courses 13-14, and is required to attend the lectures in that course. This course is spread over two terms with credit given at the end of the second term. Required of all students who declare a biology major during 1970-71 and after. Prerequisite before 1971-72, consent of Chairman; in 1971-72 and after, 28 and 29.

ELECTIVE COURSES FOR MAJORS

31 Comparative Anatomy
32 Animal Histology
33 Embryology
55 Microbiology
57 Biological Procedures
61 Comparative Animal Physiology

79 Limnology
82 Biochemistry
101 Independent Study
102 Special Studies

The Biology Core

The program of study in biology is based upon a Core of six sequential courses upon which the student builds to further his more specialized interests.

This sequence is intended primarily for students who have had a background in chemistry and who intend to work toward a biology major. The objective of the Core is to provide a general, sequential and non-repetitive foundation upon which more specialized electives can be built.

Each course in the Biology Core considers a single major topic in depth and the total sequence presents procedures and conceptions that characterize modern biological science. Except for 80, classes in these courses meet six hours per week, utilizing blocks of two or three periods at a time, so that the instructor may use the time at his discretion for best arrangement of lectures, discussions and laboratory work.

The requisite for entering the Biology Core is the student's understanding in advance some particular chemical principles, a list of which may be obtained from the Chairman of the Department of Biology. The student satisfies this chemistry requisite in one of the following ways:

1) Pre-requisite completion of Chemistry 13-14 or 23;

2) A course in high school chemistry plus co-registration in Chemistry 13-14 or 23;

3) Some other means of demonstrating this advance knowledge, which is subject to review by the Biology faculty and approval by the Department Chairman.

Guidelines for the Core

The general content of the Biology Core Curriculum will be determined by the entire Biology faculty.

Using the appropriate content guidelines, the faculty member in charge of each course will be responsible for its final organization and for all schedules, examinations, and grades.

In addition, a process of continual review will be established by the Biology faculty in order to:

1) Discuss the implementation of the established core content.

2) Make modifications in the content as necessary.

3) Develop a system of evaluation to assess the effectiveness of the core in general.

Note that the Core courses are intentionally biological. They consider principles common to all living things, and use examples appropriate to the subject at hand. This is in keeping with modern trends in biology; we now have much larger generalizations than we have had in the past.

For Non-Majors

The chemistry prerequisite for the Core would take biology out of the curriculum for most non-science majors, and the concepts of modern biology are too important a part of liberal education to be thus eliminated. We therefore established a sequence of two courses, called Biology of Organisms and Biology of Cells.

These courses are intended primarily for students who have decided to major outside the sciences, and who do not have a background in chemistry. Each course is limited in coverage of subjects, and each emphasizes certain procedures and concepts, but taken together they consider all the themes that best characterize modern biological science. Laboratory work stresses observation and experimentation with living plants and animals. Three lectures and one two-hour laboratory per week, one lecture per week treated as a conference and quiz section.

No credit from 13-14 will be given toward a major in biology. However, a student who completes 13-14 with grades of B or above, may enter 27 after satisfactory performance in a proficiency examination and with concurrent registration in Chemistry 13-14 or 23. In this case, 13-14 will count toward his major.

If a student has had college chemistry, he should be advised to take 15-16 even if he does not plan to major in biology. If he plans to take college chemistry, he should be advised to postpone any course in biology until he can meet the requirements for 15-16.

Continuous Evaluation

The attention of the reader is invited particularly to the Guidelines for the Core above, to the discussion of continuous evaluation. Our department went through a long year of arguments and eventual agreement in marathon meetings, changing our point of view about biological education, formulating new courses around the new point of view, and cancelling several time-honored courses. We are determined never to go through that process again.

We have talked purposefully with colleagues at other institutions, have heard their plans for new curricula, but none of us has been able to find a department with a new curriculum that has any but the vaguest plans for evaluation. To most of those we have talked to, even the idea of machinery for evaluation sounds contrived and unnecessary.

We have met several times this fall to put our own plan of review into operation, and have so far at least identified the instruments through which the continual review of the Core will be made: the entire faculty for the content of the courses, and the students in 80 Seminar and Teaching for the effectiveness.

As was the case in the study reported here, we feel we are traveling a new path without examples of others to follow, and the results of this study will not be apparent for at least several years, but we are determined to avoid another wrench like the one last year, and we will therefore carry it out. We feel that in biological education today there are too many new and/or core curricula going into effect without any plans to evaluate them, but, because of the difficult time we had in setting ours up, part of which fortunately grew out of the research reported herein, our new curriculum will be subject to continuous evaluation.

APPENDIX 1

Forms used to determine student's high school background.

1966-67

PLEASE CHECK YOUR HIGH SCHOOL BACKGROUND IN BIOLOGY:

_____ 1. No high school biology background.
_____ 2. BSCS Biology (Blue, Yellow or Green version).
_____ 3. Some high school biology course other than BSCS.
_____ 4. Two years of high school biology, one an advanced course.

1967-68

PLEASE CHECK THE KIND OF HIGH SCHOOL BACKGROUND YOU BRING TO THIS COURSE:

_____ A. A unit or more in Junior High School in biology.
_____ B. An entire Junior High School course in biology.
_____ C. An entire Senior High School course in biology (check kind below).

    _____ 1. A course such as BSCS Biology (Blue, Yellow or Green Version), which dealt primarily with living organisms, did not attempt to "cover the field" but inquired into the function of living things and drew generalizations from observation.

    _____ 2. A course other than the above, in which the laboratory was primarily designed around dissection and classification of organisms.

    _____ 3. A course that contained little or no laboratory but was mostly lectures and discussion.

_____ D. Two years of high school biology, one an advanced course.

APPENDIX 2

Facsimile of form used for laboratory check-in.

Locker No. ____________

FORM FOR LABORATORY CHECK-IN - FALL TERM, 1968-69

DEPARTMENT OF BIOLOGY, HAMLINE UNIVERSITY

NAME: ____

NAME: ____

I have checked out the following items from the Department of Biology of Hamline University. I understand that I will be charged for any items that are lost, damaged or broken.

Student check here if present at beginning of term.

Laboratory Assistant: check here if present and in good condition at end of term.

_____	1 Key	$ .40	_____
_____	(Second Key)	(.40)	_____
_____	1 Beaker, 400 ml.	.46	_____
_____	1 Beaker, 100 ml.	.42	_____
_____	1 Beaker, 50 ml.	.42	_____
_____	1 Brush, camel hair	.20	_____
_____	2 Culture tubes, Drosophila	.45 ea.	_____
_____	1 Cylinder, graduated, 10 ml.	.90	_____
_____	1 Dropping pipette, 10 inch	.40	_____
_____	1 Jar, quart, screw cap	.35	_____
_____	1 Loop, inoculating	.50	_____
_____	1 Pencil, glass-marking	.15	_____
_____	Pins, dissecting, about 20	.00	_____
_____	1 Rod, stirring, glass	.20	_____
_____	1 Test tube rack, wood	.40	_____
_____	6 Test tubes, pyrex, 18 x 150 mm.	.10 ea.	_____
_____	1 Test tube clamp, wire	.38	_____
_____	1 Test tube brush	.15	_____
_____	1 Thermometer, centigrade	2.00	_____

End of term check-in.
All items present and in good condition, with the exception of those crossed out.

____
Signature of laboratory assistant.

APPENDIX 3

Facsimile of form used for laboratory practicals.

Sequence No. _____

BIOLOGY ^{12w} PRACTICAL #13, 1/9/69

Locker No. _____

Score __________ Name ______________________________

1. ____
2. ____
3. ____
4. ____
5. ____
6. ____
7. ____
8. ____
9. ____
10. ____
11. ____
12. ____
13. ____
14. ____
15. ____

APPENDIX 4

Facsimile of form used for Oral Examination, Practicals 11 and 12.

GENERAL BIOLOGY 12w DATE TAKEN _____
PRACTICAL EXAMINATION 11 and 12

NAME ______________________________

I. POINTING OUT NAMED VESSELS (10) ________
____
____
____
____

II. NAMING POINTED OUT VESSELS (10)
____
____
____
____
____

III. TRACING BLOOD FROM ONE PLACE
TO ANOTHER (10)

____

TOTAL

APPENDIX 5

Facsimile of form used for oral examination, Practical 20.

Date ______ Time ______

BIOLOGY 12w PRACTICAL #20,
taken before Thursday 2/27/69 Locker No. ____________

SCORE ______________ NAME __________________________

Report turned in 1. ____________

Specimens included 2. ____________

Understanding of principles involved: 3. ____________

4. ____________

5. ____________

6. ____________

Understanding of particular cross 7. ____________

8. ____________

9. ____________

10. ____________

Other Genetics from Week #20 11. ____________

12. ____________

13. ____________

14. ____________

15. ____________

APPENDIX 6

Facsimile of one year's student evaluation form.

BIOLOGY 11 AND 12, GENERAL BIOLOGY
1967-1968
COURSE EVALUATION
(Please Fill Out Both Sides)

Do not sign this form, or indicate who you are on it. This will protect all the respondents from identification. Please fill out as much of this form as you can -- all items about which you have an opinion -- and return it at the time of the Final Examination.

A. CLASSES AND LECTURES

1. The plan for next year is to continue having a 5-minute break halfway through the lecture. Do you think this is a good plan? Yes ___ No ___ Indifferent ___
Comment: ______________________________

2. During the fall term this year, we used a good deal of (A) "team teaching", sharing the lectures on a half-hour basis. Another arrangement that we tried to some extent during the winter term was (B) "invited lectures", where one lecturer was responsible for all classes, but invited in members of the Biology faculty for some subjects. Another arrangement is (C) to have one professor give all the lectures, on all subjects. Which of these do you think we should follow next year? (A) ___ (B) ___ (C) ___
Indifferent ___

3. Would it be a good idea to close the doors at 7:45 a.m. and allow nobody to enter the class until the 5-minute break? Yes ___ No ___ Indifferent ___

4. When you come to class on time, does the late arrival of other students disturb you? Yes ___ No ___ Indifferent ___

5. In general, is class attendance important in this course? Yes ___ No ___ Indifferent ___ Comment: ______________

6. In general, have the lectures been informative enough?
Yes ___ No ___ Comment: ______________________________

7. In general, have the lectures been interesting enough?
Yes ___ No ___ Comment: ______________________________

8. It is possible to find some college level films that help to explain processes, but they are not always right on the subject, and often confuse students with theories that conflict with those presented in the course (example: "Meiosis"). With this understanding, would you like films to be a regular part of the course? Yes ___ No ___
Indifferent ___ Comment: ______________________________

9. Would you like more charts for reference, keeping in mind the size of the lecture room? Yes ___ No ___ Indifferent __

10. Would you prefer more use of projected pictures and diagrams?
Yes ___ No ___ Indifferent ___ Comment: __________________

11. Suggestions have been made that the Biology Department should institute a course for non-science majors (something like "Fundamental Concepts of Biology"), and confine 11 and 12 to biology or science majors. There are several problems involved in this area. Insofar as you can envision the problems, do you favor this idea?
Yes ___ No ___ Indifferent ___ Comment: __________________

12. a. It has been suggested that, in addition to publishing a calendar of text assignments, quizzes and practicals, we include a list of lecture topics in the calendar. How often would you use such a list of lecture topics in determining whether or not to attend class?
Always __ Frequently __ Occasionally __ Seldom __
Never __

b. How often would you use it in reviewing for quizzes?
Always __ Frequently __ Occasionally __ Seldom __
Never __

c. How often do you think we should take roll in the lecture? Always __ Frequently __ Ocassionally __
Seldom __ Never __

d. Would you like to say anything else about the lectures, not covered here? ______________________________

13. Do you think that the text, Weisz, The Science of Biology is too difficult? Yes ___ No ___ Indifferent ___
Comment: ______________________________

14. Do you think it would be valuable to have more discussion in lectures on particular parts of the text that are more important than others? Yes ___ No ___ Indifferent ___
Comment: ______________________________________

15. This year, lecture quizzes have been held as in (a) below. Assuming the same load of assignments, lectures, etc., which of the following would you prefer in addition to the essay final?

    (a) ___ Weekly quizzes, 20 points per quiz, 10 per term.
    (b) ___ Quizzes every two weeks, 40 points per quiz, 5 per term.
    (c) ___ Quizzes every three weeks, 66 points per quiz, 3 per term.
    (d) ___ A midterm and a final. 100 points per quiz.
    (e) ___ One final, 200 points.
    (f) ___ Indifferent.

16. Do you know of any instances of cheating in lecture quizzes? Yes ___ No ___. If "yes" how many instances could you cite? ______________________________

17. Do you think the essay type of final examination is the best type for the course? Yes ___ No ___ Indifferent ___
Other comments about the classes and lectures? __________

B. LABORATORY

18. The alternative to Open Laboratories is two two-hour laboratory periods scheduled per week, with the laboratory continuously occupied. Do you prefer Open Laboratory? Yes ___ No ___ Indifferent ___

19. Laboratory practicals were given weekly this year, Do you think it would be better to have them twice as long, but given every other week? Yes ___ No ___ Indifferent ___

20. Do you like this year's system of having students take the laboratory practical at any time that they are ready, unscheduled? Yes ___ No ___ Indifferent ___

21. Do you think there should be some non-practical questions included in the lab practicals? Yes ___ No ___ Indifferent ___ Comment on practicals: ______________

22. The photographs in the laboratory manual were new last year. Their purpose is to help the student find things and demonstrate procedures. Do you think they actually achieve their purpose? Yes __ No __ Comment: __________

23. General comments about the laboratory manual: ____________

24. Please evaluate the following laboratory weeks, on the basis of their value to your biological education. Mark (X) in five spaces in each column, most valuable to the left, least valuable to the right.

MOST		LEAST
_____	1. Materials and Methods	_____
_____	2. Biological Chemistry	_____
_____	3. Monera	_____
_____	4. Animal-like Protista	_____
_____	5. Plant-like Protista	_____
_____	6. Porifera, Cnidaria	_____
_____	7. Platyhel and Nematoda	_____
_____	8. Annelida and Arthropoda	_____
_____	9. Mollusca, Enchino	_____
_____	10. Vertebrate Structure	_____
_____	11. Arteries of a Bullfrog	_____
_____	12. Veins of a Bullfrog	_____
_____	13. Mesodermal Derivatives	_____
_____	14. Gross Morphology	_____
_____	15. Epithelium	_____
_____	16. Mitosis and Gametogenesis	_____
_____	17. Metazoan Embryology	_____
_____	18. Non-flowering Metaphytes	_____
_____	19. Angiospermae	_____
_____	20. Genetics	_____

25. Is there any part of the laboratory (whole week or part of any week) that you would like to see eliminated next year? Yes ___ No ___ Indifferent ___ Comment: ____________________

26. Is there anything that we should add to the laboratory that is not included? Comment: ____________________________

27. As you understand the course to be planned next year, would you recommend it to students to satisfy a distributive requirement? Yes ___ No ___ Indifferent ___
Comment: ____

3.10

OPEN LABORATORY 1

Third Edition

A NEW APPROACH
TO COLLEGE GENERAL BIOLOGY
LABORATORY STUDIES

First Term of a Two-Term Course of Study

William L. Downing, Ph.D.
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Hamline University

Published by the author at Hamline University, St. Paul, Minnesota 55101

OPEN LABORATORY 1

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Introduction

I. EQUIPMENT REQUIRED.

All the items listed below are required for Biology 11 General Biology, and are available from the Hamline University Bookstore. All but this manual will be used during Biology 12 as well.

1. A copy of this book, Open Laboratory 1.
2. A copy of the text, Weisz , Paul B., The Science of Biology, edition 3, 1967, McGraw-Hill Book Company, New York.
3. A bound, cross-lined laboratory data book.
4. A dissecting kit of good quality.
5. A packet of lens paper.
6. About a dozen microscope slides and cover glasses.

It is recommended that you also have the following:

7. Plain white paper for laboratory reports.
8. A copy of the book Brenner, Robert M., Study Guide for Weisz: The Science of Biology, edition 3, 1967.

II. THE COURSE.

This is a unified, single course in introductory biology, emphasizing molecular biology and using an open, voluntary type of laboratory attendance. The course was designed by Hamline University biology faculty to fit into Hamline's 3-3 program. In setting up this type of course, we had the following objectives:

1. To present a single introductory course, rather than a group of courses such as General Zoology, General Botany and General Biology. It has only been recently that such a course has been made possible, by the great advances in biological knowledge and the synthesis of this knowledge into a few broad principles rather than a scattering of isolated facts.

2. To give you the best possible background in biology, by teaching the subject as a science, and by having you learn scientific techniques and scientific point of view.

3. To save time for you, by avoiding repetition of high school work, and to provide space for you in the laboratory when you need additional time for your work.

We began this system of Open Laboratories in the fall of 1964, and have had enthusiastic approval from the students taking the course during the years it has been in operation. Such a system requires thoroughgoing modifications in traditional laboratory methods, and most of the changes have worked out very well. At present we are conducting an evaluation of the Open Laboratories under a grant from the

U.S. Commissioner of Education under the provisions of the Public Law 531, in the hopes that this course at Hamline may be an example for other colleges to follow in setting up open laboratories of their own. Because of this evaluative study, we ask that you keep careful notes on the time cards of the time that you spend in the laboratory (see Part V below), and when you are asked to fill out evaluation forms for the course, that you will do so to the best of your ability. Biological education in many colleges may be affected by what we do at Hamline now.

III. THE LECTURES.

Held third period, M-W-F, S-118. Attendance is required, seats will be assigned, and roll will be taken. Lectures have the following purposes:

1. Explain and illustrate laboratory work.
2. Review and summarize laboratory data, and connect it with other biological information.
3. Present new material not covered in laboratory manual or text.
4. Explain some ideas in the text.

The 70-minute lecture period will normally be divided into two sessions, usually with a five-minute break between them. All members of the biology faculty are cooperating in the presentation of this course, in order that you can have the most up-to-date information by the most appropriate departmental expert in his particular field of biological specialization.

You must learn early in the course to listen and watch the lecturer intelligently, and to keep good lecture notes. Most freshmen try to take too many notes, and often miss other points that are brought out. It is best to take brief notes in class, and expand them later if necessary.

Frequently the lecturer will bring up topics that appear to be unrelated to text assignments or the laboratory. This is necessary in order to give proper background for future work, and their connection with the rest of the course will eventually become apparent. All parts of the course are inter-related, but the relationships are not always instantly evident.

IV. THE TEXT.

Weisz, Paul B., The Science of Biology, edition 3, 1967.

The text assigned is an excellent accessory reference for the lectures, and that is how we will use it. Your weekly assignments are given in the Calendar which will be distributed during the first week of classes, and must be either read or studied when assigned. Lecturers will attempt to point out the relative emphasis to be placed on various parts of the text, but we will proceed on the assumption that you have at least read the assignments before hand. A good study help is provided in Brenner, Robert M., Study Guide for Weisz... Many students have found this volume to be an invaluable aid to their studying.

V. THE LABORATORY, S-203.

Laboratory assignments are made on a week-by-week basis, and the work must be completed during the week assigned. Come to the laboratory at any time you wish during the laboratory week. A faculty member or laboratory assistant will be present during all hours that the laboratory is open to help you with your work.

When you come to the laboratory, take your laboratory card from the rack and mark it with the date and time in; and when you leave, mark the time out. It is important that we have these data in planning for work in future years.

The responsibility for completing your laboratory work is placed upon you. You must make sure that you are familiar with all material presented for the week; that you have made all the laboratory records that are required; that you have the data for any reports assigned.

VI. LABORATORY CHECK-IN.

You must carry out this process during the first week of class. The purpose is to assign you a locker and check out its contents to you. You may have a locker entirely to yourself, and bear the sole financial responsibility for any breakage that occurs; or you may share a locker with one other person of your own choice, and divide any breakage charges between you. If you plan to share a locker, you and your prospective lockermate must report for check-in at the same time, and both of you will have a separate key to the locker.

For checking in, go to the faculty member in charge of the laboratory, and have a locker assigned. He will give you a key and a check-in slip. Open the locker, identify all the equipment in it, and make sure it is all in good condition. Then sign the slip and give it back to the faculty member.

During your last week in the course you must reverse the process, and check out of the laboratory. The cost of any lost, broken or damaged equipment will be reported to the Business Office for collection.

VII. LABORATORY RECORDS.

Think of your laboratory data book as a diary of everything you do and discover in the laboratory. Never keep lab records on any other paper! The form of your records is up to you. The virtue that you should pursue in lab records is accuracy, not neatness nor beauty. If you are accurate, neatness will follow.

The first thing you must do each time you open the book is to enter the date and the time. Make all entries in ink. Write down, in some form, all your observations. The form may be a list, a description, a sketch, a table, a graph, etc. Never tear out a page from your data book. You may cross out sections or words, but never cross out anything so you cannot read it later.

Any information may be included in this book, in any order, but you must avoid plagarism. If the information is not of your own observation, you should record it in a different color of ink, and make a note at the time of its source -- another student, an assistant, a book, etc. This will help you when the time comes for writing reports.

Data books will be examined by the Biology Faculty from time to time.

VIII. REPORTS.

Think of reports as finished products. A report is an assembly of information taken from your data book and other sources, compiled and condensed into a readable form and ready for publication. Reports are factual, and are never filled with excess wordage. Reports should be typed; but if you cannot type, then you must have a sample of your handwriting approved by your conference instructor before submitting handwritten reports.

Keep in mind as you write a report, the main purposes they have:

1. To tell other scientists what you have observed.

2. To allow other scientists to repeat your work and observations.

All reports must have the following clearly-labeled sections:

Title: a few words telling what the report is about.

1. Introduction. If your work came from a hypothesis (and most scientific work does), state it here. If you have read books or articles that have led up to the work, here is the place to say so. If the work is based upon any assumptions that are not self-evident, this is where you should state them.

2. Materials and Methods. All published research papers in biology contain this section. Its value lies in making the work you have done repeatable by any other person who wants to try it. If you followed procedures listed in some publication, you should say exactly what and where, and put that publication in your list of references. If you invented your own procedures, you should describe them clearly. If you modified someone's published procedures, you should describe the modifications you made. You should list all unusual laboratory equipment or material that you used. You need to use good sense in this section. Do not copy procedures that can be found easily elsewhere. If you look at it from the point of view of the reader who is trying to repeat your work, you won't go wrong.

3. Observations. This is the place for your data which might be in the form of descriptions, tables, graphs, etc. You must never include any interpretation of your data in this section. Check for pertinence this way: "Is this what I really saw (heard, smelled, tested, felt)?"

4. Interpretation. You might think of this as the "conclusions" you make from your data; but since conclusion means ending, and most data lead not to an ending but to another beginning, interpretation is a better word. If your observations supported your hypothesis, this is the place to say so. It is here that you state what your observations mean.

5. Discussion. An optional addition to your report, which might show how your discoveries fit into other discoveries or theories; or what your colleagues in the laboratory have discovered; etc.

If your work raises new questions, state them here. If you feel you might have done better with modified procedure, say so here.

6. References. Never omit this section -- all research comes from somewhere, and this is where you give the credit. Also, remember the repeatability principle: your reader must know where your methods came from. Alphabetize by author or source person; number all the entries, and then in the body of the report you only need to put the number of the entry in parentheses ( ) to refer to it. You should avoid the use of footnotes in a scientific report unless you find one to be absolutely necessary.

Use the following form for your entries:

(1) DeRobertis, E.D.P., W.W. Nowinski and F.A. Saez: General Cytology, edition 3, 1960.

(2) Smith, John E., personal communication, January 10, 1966.

(3) Uhr, Jonathan W., "The Heterogeneity of the Immune Response", Science, Vol. 145, pp. 457-464, 1964.

IX. CONFERENCE SECTIONS.

You have registered for a conference section that meets on Tuesday during some hour. Your attendance is voluntary; the discussions are informal and helpful. The place of meeting, and the conference leader, will be announced in the lecture during the first week of classes. Topics for discussion at the conferences will be announced by the conference leader, and his decisions will be guided by student requests.

X. TESTS.

1. A Lab Quiz will be given every Thursday. You may take this quiz at any time during the day, up until 4:00 p.m. The lab quiz is designed to test what you have retained and learned from the previous week's laboratory work. Most of them will be of the practical, or demonstration type.

2. A Lecture Quiz, 15-20 minutes in length, will be given at the beginning of every Wednesday lecture period, as listed in the Assignment Calendar. This quiz is designed to test what you have retained and learned from the previous week's text readings and lectures. Many of these will be of the single-question discussion type.

3. The Final Examination is a two-hour objective type designed to see how you have assimilated the general principles presented during the preceding term.

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11 pages, green.

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14 pages, white.

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LABORATORY WEEK 1:
MATERIALS AND METHODS

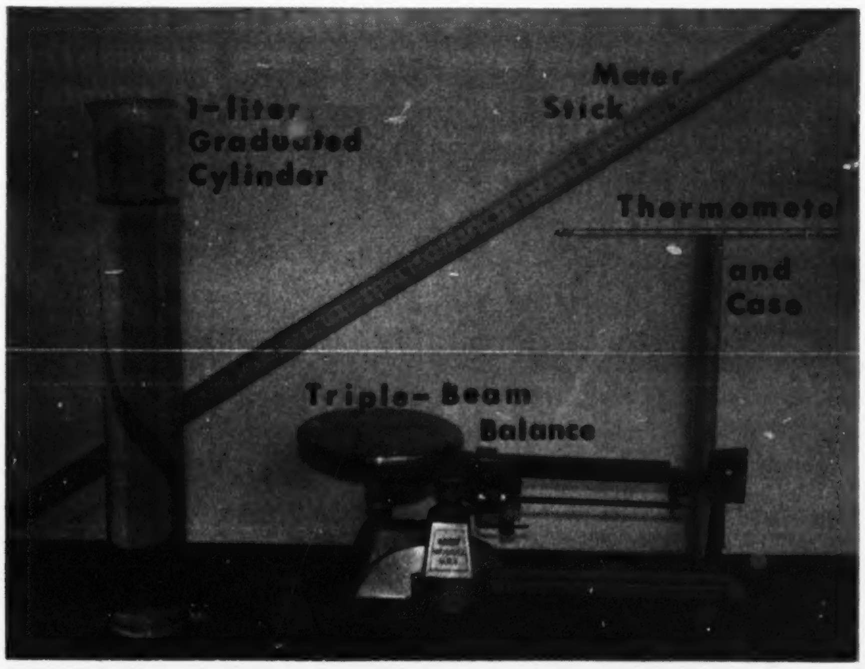
Purpose: to make sure that all members of the class have an adequate background in the equipment used in biology and its proper use. If you have had an excellent high school course in biology, then you will find this week's work short and easy; but if you have had little or no exposure to biological methods in the laboratory, you will find much more in the week's work than you can do in any way except by putting in many extra hours.

You will be held responsible for all the material in this week's work, but you should plan only to follow those parts of the directions that you feel you need to; depending upon your previous experience in biology.

I. METRIC SYSTEM

All measurements made in this course must be made in metric units, since nearly all science is based on this useful international system. No doubt in the past you have been introduced to meters, liters and grams, but have probably been mostly concerned with their English equivalents rather than being concerned with their relationship with each other. The following should help you visualize the units.

Fig. 1.1 Some useful measuring equipment with which you may be unfamiliar.



A. LINEAR MEASUREMENT.

1. Examine a meter stick. The meter (m.) is divided into ten decimeters (dm.); each decimeter is divided into ten centimeters (cm.); each centimeter is divided into ten millimeters (mm.). How many centimeters in a meter? How many millimeters in a meter?

2. Measure your height on the physician's scale, and make sure you understand what the units of measurement are.

3. Find some convenient part of your hand that is about 1 cm. in length, so you can use it in estimating the sizes of things when you have no meter stick. Do the same for a decimeter.

B. VOLUME MEASUREMENT.

1. Examine a 1-liter graduated cylinder. Pour a quart of water into it and see how full it makes the cylinder. The liter is one thousandth (1/1000) of a cubic meter, or one cubic decimeter. Look at the paper box labeled 1 dm. on each edge, in order to visualize the liter better.

2. Fractions of a liter may be spoken of in the same way we speak of fractions of a meter: deciliter, centiliter, milliliter. How much of a liter would each of these terms indicate? Which of these units is assumed in the graduations and numbers on the 1-liter graduated cylinder? We usually refer only to the milliliter (ml.) in writing and speaking. How many ml. in a deciliter? In a centiliter?

3. One of the most useful relationships for you to remember is that one milliliter equals 1 cubic centimeter (1ml. = 1cc.). Older literature speaks of the cc. ("see-see") while newer literature speaks of the ml. ("mill"). This relationship, plus what you learned in B-1 above, makes it possible for you to interchange linear and volume measurements. For example: Try to decide by inspection how many liters the aquarium in the laboratory would hold; then measure it in decimeters and determine the figure precisely. Or measure it in centimeters and determine how many cc. (=ml.) it would hold. If you had a ruler marked off in inches, could you determine easily how many quarts of water it would contain?

4. From a distance, look at the group of beakers and flasks on the front table, and try to decide by inspection which of them would hold a liter. Then check your judgment by looking at the labels on them.

5. A very useful rough measurement (useable in the second laboratory week and following) is that 1 ml. is equal to about 20 drops from a dropping pipet. Check this statement, using tap water and a small graduated cylinder. Check it again after adding about 1 ml. of dishwashing detergent to tap water. Check it again with ethyl alcohol instead of tap water. How accurate does this rough measurement seem to be?

C. WEIGHT MEASUREMENT.

1. By definition, using earth's gravity, one ml. (=cc.) of pure water at 4°C. will weigh one gram (gm.). This unit is so small that, for our purposes in laboratory, fractions of it are not used. However, biochemists often use the milligram (mgm.) which is one-thousandth of a gram. Multiples of the gram are used more commonly, especially the kilogram (kgm.) which is one thousand grams. Weigh yourself on the physician's scale, and make sure you know what the units of measurement are.

2. Using the triple-beam balance, weigh several common articles in grams. Then try to guess the weight of some other articles before weighing them. With practice, you should be able to guess weight within a few grams.

3. Weigh a small beaker accurately, then add 10 ml. of tap water and weigh it again. The difference between the two weights is the actual weight of the water. How much do you expect the water to weigh? Several factors may bring about differences from the expected----what are they? How much should the water in the aquarium weigh?

D. TEMPERATURE MEASUREMENT. On the centigrade (means "hundred-place") thermometer, 0°C. (zero degrees centigrade) is the freezing point of pure water under particular conditions, and 100°C. is its boiling point. Examine the centigrade thermometer provided, find 0° and 100°, and see how it is marked off. Determine the temperature of some common reference points that you can remember, such as room temperature, ice, boiling water, human body temperature, tap water, etc.

E. HEAT MEASUREMENT. It is essential that, at the beginning of a course in biology, you separate in your mind the terms "heat" and "temperature." Heat is a particular kind of energy given off by materials; temperature is the effect of this heat on a thermometer. Many kinds of materials will absorb rather large amounts of heat into their structures without much change in their temperature; other materials show a temperature change after absorbing very little heat.

By definition, one calorie (cal.) is the amount of heat required to raise the temperature of one gram of pure water one degree centigrade, under particular conditions. We do not have the time to carry out careful experimentation with this concept, but you can at least carry out a gross demonstration of it. Put 100 ml. of water into a beaker, place the beaker on wire gauze on a ring stand, and measure its temperature. Then put a very low flame from the propane burner under the gauze, stir the water constantly, and see how long it takes to raise the temperature by 10°C. When finished, determine how many calories of heat you have added to the water.

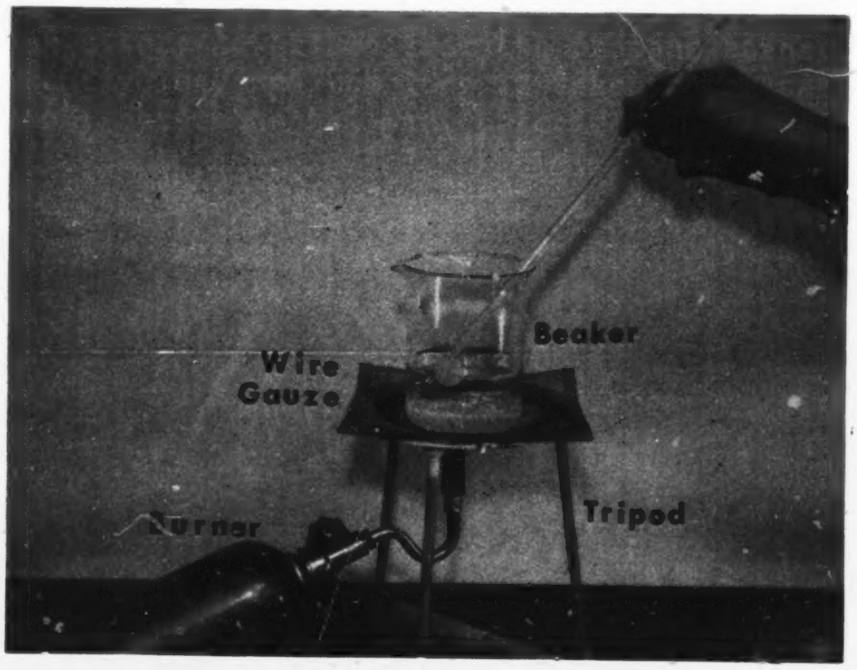


Fig. 1.2 Equipment used in heating water and other non-flammable liquids.

Repeat the above with another liquid substance or solution such as salt solution, sugar solution, corn oil, etc. Duplicate the conditions as well as you can, and compare the amount of time required to raise the temperature 10°C. If the temperature rises more slowly than it did with water, then it must mean that more heat is being taken up per degree of temperature change. Make an interpretation of your data, with regard to the heat capacity of the substance you used, compared with the heat capacity of water.

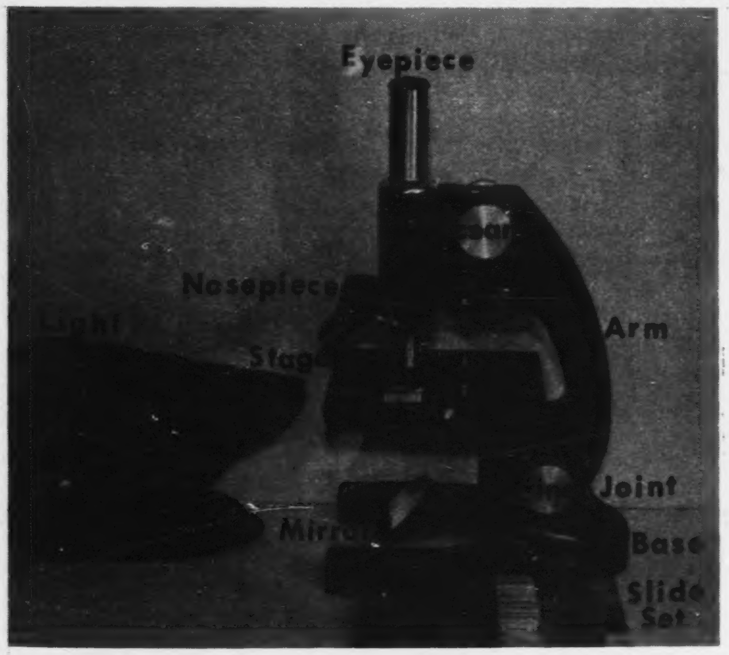
(Note on the term "calorie": The calorie we discuss here is not the calorie of nutrition. That one is really a kilocalorie, 1000 times larger than our calorie. The kilocalorie was once improperly abbreviated "Cal.", but is properly abbreviated "Kcal.", and that is the source of the unfortunate double use of this useful word.)

II. COMPOUND MICROSCOPE

The microscope has been described as "the most important tool of modern biology," since without it we would be unable to see the fine structure of living things, and it would be impossible to understand them even as well as we do. Therefore, in this beginning course in biology, you must learn to use the microscope properly and with maximum efficiency. Too many students try to barge ahead without adequate learning, and as a result do poorly because they do not see what they should see.

A. WHAT THE MICROSCOPE DOES. A good microscope is a precision instrument designed to change the direction of light rays, in order that the viewer may see an enlarged view of the object which is on the stage. This is accomplished by directing the light through a series of glass lenses enclosed in a tube. The material to be examined must be thin enough so that the light can pass through it; but at the same time it must be dark enough to interrupt some of the light, otherwise nothing but light would be seen.

Your microscope will be kept in the cupboard beside the kneehole of your desk. Note that the cupboard has a number; the microscope has the same number; the microscope light also has the same number; and the box of slides in the cupboard, and the slides in it, are numbered in the same way. Always put these things away in the right.cupboard when you are through using them!



← Fig. 1.3
Compound microscope.

Fig. 1.4 →
A slightly different type of compound microscope used in our laboratory. Note position of the fine adjustment.

You should be able to refer to the parts of a microscope readily:

The whole instrument rests on a base, and extending up from that is the pillar which supports the remainder. The entire upper part of the microscope is held by the arm, which swivels around an inclination joint. Light is directed upward by the mirror, through the condenser (if there is one), which focusses or concentrates the light on the glass slide holding the material you are examining. The slide rests on a large square stage. Just under the stage is a diaphragm, used in adjusting the amount of light that reaches the slide. Above the stage is the round revolving nosepiece, into which are screwed two or three objectives which are lenses. Notice that the objectives can be turned into place, and when properly in place they click. Objectives are usually 10x low power, and 43x high power. Extending upward from the nosepiece is the body tube, at the top of which is another lens-group, the eyepiece. The entire body tube with its nosepiece and eyepiece can be raised or lowered rapidly by the large knob, the coarse adjustment, or slowly by the smaller knob, the fine adjustment.

B. TO PROTECT THE (VERY EXPENSIVE) LENSES, TAKE THESE PRECAUTIONS:

1. Use only lens paper for cleaning lenses. Use it once, then discard it.

2. Never touch lenses with your fingers. Never let them get wet.

3. Never focus downward without watching the objective. Going too far will put the objective through the slide, spelling disaster for both slide and lens.

4. Watch the objectives while changing from low power to high power.

5. Lenses are cemented together and cemented into their mountings. Therefore, never take a lens apart, and avoid dropping lenses. A slight jar can completely ruin one.

6. When making slides of wet material, take special pains not to get the lenses wet. Avoid excess fluid on slides, and use a coverglass on all preparations.

C. TO PROTECT YOUR EYES, TAKE THESE PRECAUTIONS:

1. See to it that the lenses are always clean.

2. Never attempt to study an object until you have centered the light properly by means of the mirror. The field should be lighted brightly but not too brightly.

3. Keep both eyes open! Most eyestrain is caused by trying to squint one eye closed.

4. Change the focus using the coarse and fine adjustment, not by trying to focus with your eyes. You cannot do it in any case; but constant trying will exhaust you.

5. Never start an examination of a new slide under high power. Always start on low power and stay on low power until you must change to high.

6. Use nothing but clean slides and coverglasses. Clean off fingerprints and dirt before you start with a slide. Handle prepared slides by the ends, never by the middle, and avoid fingerprints.

D. APPEARANCE UNDER THE MICROSCOPE.

1. Clean the lenses and mirror with lens paper, discard the paper.

2. Take Slide #24 out of your slide box. Inspect it with the naked eye, then put it on the stage with the coverglass uppermost. Hold the ends of the slide down with the clips. Center the printed letter on the slide in the middle of the hole on the stage.

3. Turn the low power (10x) objective into position, and lower it with the coarse adjustment until it is within about 6 mm. of the slide.

4. With the mirror, center a beam of light upon the object. By looking from the side, you can see when the letter becomes brighter due to the light.

5. With your eye at the eyepiece, raise the tube slowly, using the coarse adjustment knob. Move the slide, if necessary, until it is centered. Finish focusing with the fine adjustment.

6. Close the diaphragm until the intensity of light is just enough to reveal details.

7. Record the appearance of the letter as compared with its appearance to the naked eye. Is the letter rotated? Is it reversed? Move the slide slowly, and see which way the image moves.

E. ESTIMATION OF THE DIAMETER OF THE MICROSCOPIC FIELD.

The purpose of this is to mount an actual millimeter on the stage of the microscope, and see how the diameter of the field (the bright circular area you see under the scope) compares with that millimeter.

1. Mount your plastic ruler on the stage so you can see two of the millimeter markings on it, and the space between.

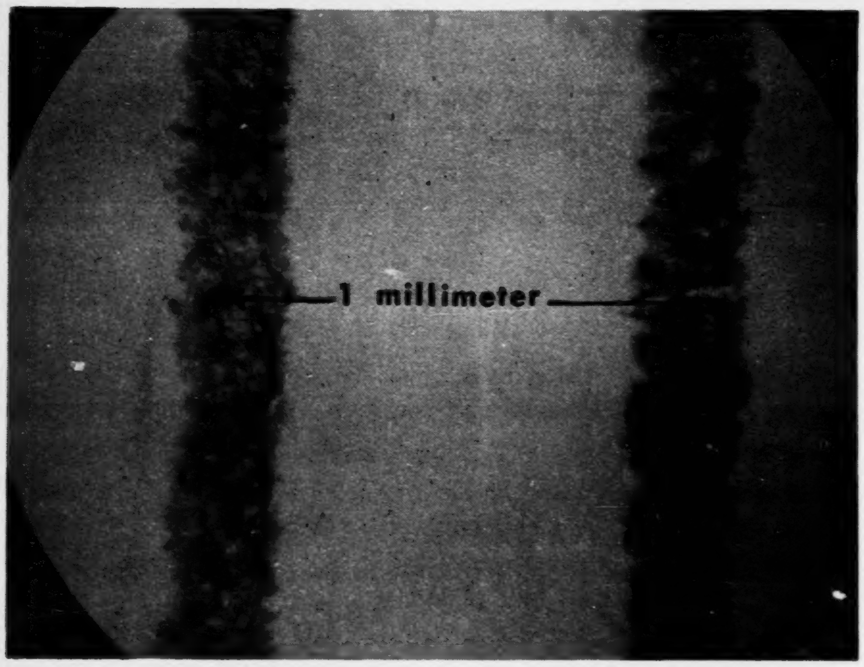


Fig. 1.5 One millimeter on a plastic ruler, as seen under the low power (10x) objective.

2. Since you can actually observe the one millimeter under the microscope, it should be easy to estimate about how many millimeters it would be across the entire field of the microscope. Do so, and record as "diameter of the field in millimeters."

3. There are 1000 microns in 1 millimeter (1000 µ = 1 mm.). Compute and record the diameter in µ.

4. Turn to high power and examine the lines. Note that you cannot see the individual lines well enough to make an accurate measurement. The assistant will give you the usual figure for the diameter of the high power field.

F. ESTIMATION OF SIZES OF THINGS SEEN UNDER THE MICROSCOPE.

1. Leave the plastic ruler or graph paper in place under the microscope. Borrow an ocular micrometer eyepiece from the assistant, and use it in place of the regular eyepiece in your microscope. This eyepiece contains a disc of glass with a numbered scale etched onto it. Note that this is an arbitrary scale, and will not change magnification when you change powers at the nosepiece.

1 mm. = 1000 µ
10 arbitrary units

Fig. 1.6 One millimeter on a plastic ruler, as in 1.5, but with ocular micrometer eyepiece. The micrometer that you will see in your microscope will not look exactly like this.

2. Assume for a moment that you see the above in your microscope. If the arbitrary scale of the ocular micrometer extended exactly between the two markings of the 1 mm. or 1000 μ, and there are 10 units on the scale, then how many microns would be included within each unit?

$$\frac{1000\ \mu\text{ measured}}{10\text{ units}} = 100\ \mu/\text{unit.}$$

Carry out this same kind of work under your own microscope, determining about how many units on the ocular micrometer it would take to extend across 1 mm. (1000 μ). For low power, you will probably get a figure between 50 and 150 μ per unit.

3. Under high power, you cannot see a whole millimeter, so estimating the number or microns per unit is more difficult. Estimate this to the best of your ability, however, and especially notice that the figure will be different under the different power. Would you expect the number to be larger or smaller?

4. Another, and rougher, method of estimating size is to use the diameter of the field. Suppose that you were looking at a cell under the high power, and you knew that the diameter of the field was 400 μ. By inspection, you decide that it would take 20 cells of this size to extend across the field. Then about how many microns in diameter is the cell?

$$\frac{400\ \mu}{20\text{ cells}} = 20\ \mu\text{ per cell}$$

5. As a general rule, you should always make a record of the size of things you examine under the microscope. Exact sizes are not necessary, but a good rough estimate is absolutely essential! Therefore, in all

future work, never sketch anything or make records of anything examined microscopically without including a record of its estimated size.

G. MAGNIFICATION. Students are often confused about what the magnification of a microscope might be. It is simply the apparent size of something after it has been magnified. For example: If a line 1 mm. long is magnified so it seems to be 100 mm. long, then its magnification is one hundred times (usually written 100x). The magnification of a lens is usually marked on it. Examine the tube of the low power objective, and find the inscription "10x" on it. What does that mean? Examine the eyepiece, and find the inscription "10x" on it also. If the material on the slide is magnified ten times by the objective, and then that image is magnified ten times again, then what is the magnification of the image that you see? Look at the high power objective, and determine its magnification. What is the magnification of the image that you see under high power?

H. VERTICAL PLACEMENT ON THE SLIDE. Put Slide #25 from your slide box onto the stage of the microscope. This is a sandwich of colored threads and coverglasses, made in such a way that each thread is separated from the next by the thickness of a coverglass. DO NOT USE HIGH POWER ON THIS SLIDE: IT IS TOO THICK. By focussing upwards and downwards, determine which one is on the low level, which is in the middle, and which is on the high level. Get used to the action that you must use in focusing to bring higher and lower things into clear focus.

J. APPEARANCE OF LIVING CELLS UNDER THE MICROSCOPE.

1. Lightly scrape the lining of your cheek with a clean toothpick, and then stir it in 3 drops of saline solution in the middle of a clean slide. It will not look as though you have obtained anything, but you will, because the lining of your mouth is continuously sloughing off old cells as new ones are formed.

2. Drop a coverglass over the drops of saline, and examine using faint light under low power. The cells will look somewhat like those below. The cells are flat; and although you will see most of them as roundish bodies, you should be able to find a few in edge view.

Fig. 1.7 A large number of sloughed human cheek cells, as seen with faint light under low (10x) power. A few of the cells are indicated with arrows.

3. Examine and sketch the cells under both low and high power. Do not neglect to estimate the sizes of the individual cells!

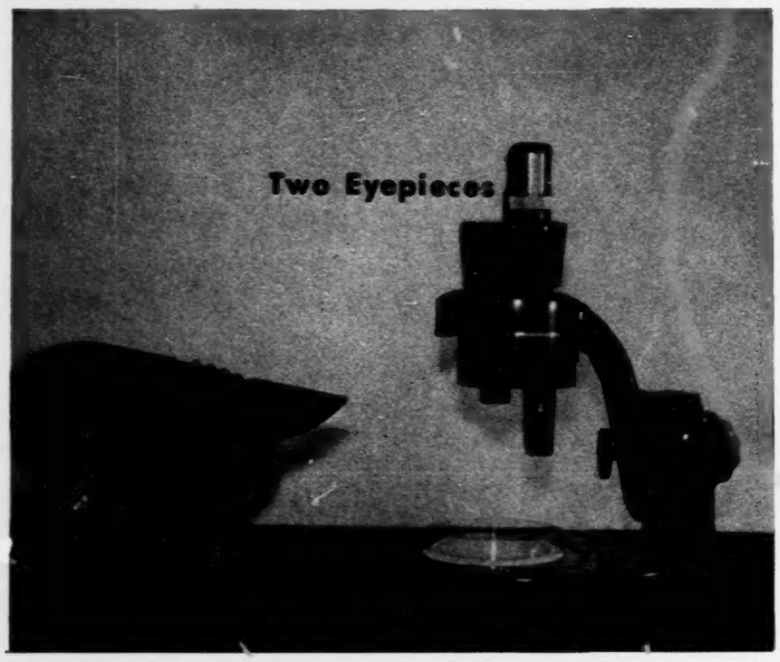
4. Put a drop of methylene blue dye on the slide next to the coverglass, and let the dye diffuse across the preparation. Which parts of the cells take up the dye the most rapidly? Which parts do not seem to take up any dye at all?

5. For further practice in making wet preparations, if you wish: try making slides of a piece of sloughed skin from a frog, and/or one thin leaf from an Elodea plant. Feel free to try using any of the various vital dyes that you find on the front table.

III. BINOCULAR DISSECTING MICROSCOPE

Become familiar with this other useful optical tool, following the general procedure outlined above for the compound microscope. Determine its magnification, the diameter of its field, and make sure you understand how to adjust it for the distance between the pupils of your eyes.

Fig. 1.8 Binocular dissecting microscope. Adjust for your interpupillary distance by rotating the two eyepieces either toward or away from each other.



IV. MAKING AND RECORDING OBSERVATIONS OF A LIVING ANIMAL

A great deal of scientific activity is performed by simple observation--biology is not by any means just dissection and test tubes. But observation includes not only looking, but knowing what to look for, how to look for it, and how to record it. Science really boils down to this--how to ask the questions of the material studied, that will yield fruitful answers.

For this section, use a common leopard frog, Rana pipiens, observe it from many viewpoints, and practice recording everything carefully. This section will also give you quite a bit of terminology that you will need, both in making accurate notations and in dissecting a bullfrog later in the course.

A. POSITION AND ORIENTATION.

Fig. 1.9 View from dorsal side of grass on Leopard frog, Rana pipiens.

1. Place the frog on the table and observe its normal resting position. The frog will hold still for long periods if you do not make sudden motions or loud sounds.

2. The frog is divided into head, trunk and limbs. It has no neck or tail.

3. The head, or cephalic end of the frog usually goes foremost in locomotion, and is therefore called the anterior end; while the other or caudal end, hindmost in locomotion, is called the posterior end.

4. The dorsal surface is directed upward when the frog is in its natural position, and the ventral surface is directed downward.

5. There are right side and left side, with corresponding parts, and the line between the right and left is termed the midline of the animal. With reference to this imaginary line, any part is called proximal if it is near the line and distal if away from the line (for example, the arm is proximal to the hand, the hand is distal to the arm).

B. THE SKIN.

1. Note and record the coloration of the skin: what colors, how arranged. Is there a definite pattern carried from frog to frog? How much variation does there appear to be? How does the frog's coloration adapt it to its environment?

2. Feel the skin, and record accurately its texture, moisture content, slime, hair or feathers, claws, etc., on it.

C. THE HEAD.

1. Observe the head. What is its shape? In comparison with the head of a human, is it relatively small or large with reference to the body? Can it be rotated on the body? Describe the location and size of the mouth.

2. Study the eyes as to position and appearance. Look through the front of the eye. Its front covering is transparent and is called the cornea. Behind it is the gold flecked iris, whose central opening is the pupil. There are three eyelids: upper, lower and nictitating membrane. Can the frog blink its eyes? Explain. Try to determine the area of the frog's vision. The frog's normal food is flying insects. Can you see what advantage there is in the placement of its eyes?

3. Locate the nostrils near the anterior dorsal end of the head. The external nares (the technical name for these openings) can be closed with valves, and this closing is essential to breathing. How do the valves work? Observe very closely the breathing of the frog. You may have to wait a while--frogs can stop breathing for long periods and carry out gas excahnge through the skin only. By observing the nostrils, floor of the mouth and the sides of the trunk, you should be able to conclude exactly how the frog gets air into and out of its lungs.

4. The eardrums (tympanic membranes) are naked in the frog. That is, there is no external ear such as you have. They are situated flush with the skin behind the eyes.

5. Look for the brow spot, a small dot on the skin between the eyes. This marks the vestige of a third, median eye.

D. THE TRUNK.

1. Two dorso-lateral ridges extend posteriorly from the eardrums. The skin is thickened in them by the presence of glands. It is possible that these ridges function in detecting changes in water pressure.

2. The anus, the posterior opening of the digestive, urinary and reproductive systems, is located on the dorsal surface between the bases of the hind legs. (This opening is more accurately termed the "cloacal pore," rather than anus.)

3. The prominent hump in the middle of the back of a sitting frog marks the junction between the hip bones and the spinal column. Does it appear to be flexible?

E. THE LIMBS.

1. The frog possesses four limbs: two fore-limbs and two hind-limbs. Note that although these have similar parts, they are of different sizes and have different functions. The parts of the limbs are--

Fore-limb:	Hind-limb:
Upper arm	Thigh
Lower arm	Shank
Wrist	Ankle
Hand	Foot
Fingers	Toes

2. Observe how the frog uses its limbs, hands and feet. Can it grasp? Can you find any webbing on either limb? How many fingers and toes does it have?

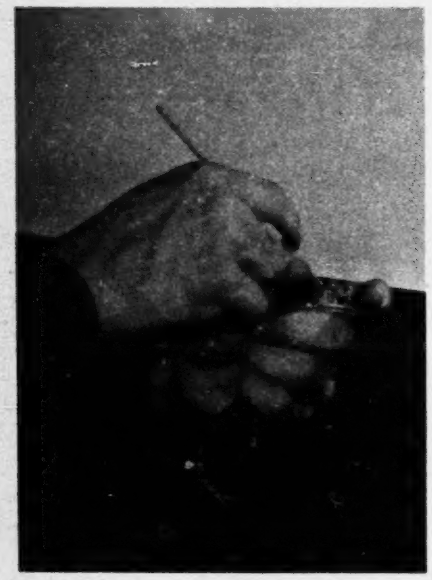
3. If your specimen is a mature male, find the enlarged "thumb" on the fore-limb. Be sure that you can distinguish between male and female frogs.

V. INTRODUCTION TO DISSECTION

The purpose of this section is to familiarize you with your dissecting instruments and to get you used to fresh material--much different from the stiff bodies of preserved specimens. Regions and organs of the frog learned here will be helpful when you dissect a bullfrog, later in the course. Please refer to your dissecting instruments as scissors, forceps, dissecting needles, scalpels; not by such names as tweezers and knives.

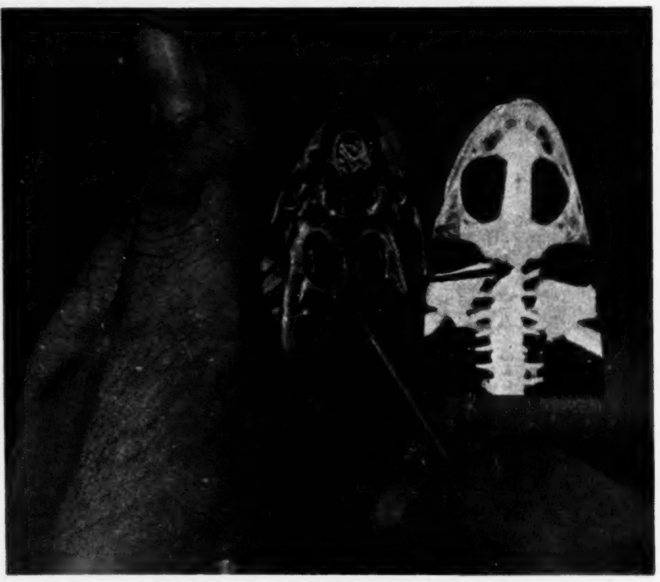
Dissection is an aid to observation, not an end in itself. Observation should accompany dissection, not left until later. Dissection allows you to get inside an animal or plant, allows you to separate parts from each other, so you can see better or more clearly. Always remember the original relationship of the parts to each other. Carry out all the cutting a little at a time. Remove parts only when you are sure you can put them back in their same relative position. Observe everything as you go along; don't chop now, and look later.

A. KILL THE FROG BY PITHING IT. The instructor will help you in finding the foramen magnum, which is the rear opening of the brain case.

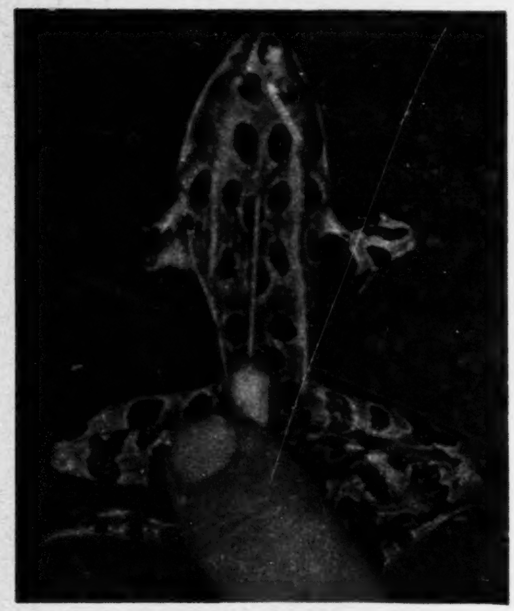


←Fig. 1.10 Pithing. Bend head forward.

Fig. 1.11 Pithing→ Needle is in foramen magnum. Arrow on skeletal inset shows location of foramen magnum.

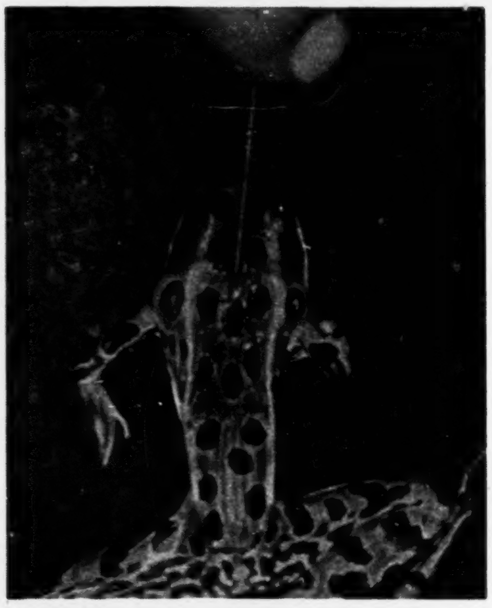


Insert a needle forward into the brain case, and stir the brain thoroughly.



←Fig. 1.12 Destroying the brain.

Fig. 1.13 → Destroying the spinal cord.



Then insert the needle backward through the neural canals of the vertebrae and stir the spinal cord. This procedure is the quickest and kindest way of killing the frogs, and leaves everything but the central nervous system in excllent condition for observation.

B. DISSECTION DIRECTIONS.

1. Place the pithed frog ventral-side-up in a paraffined dissecting pan.

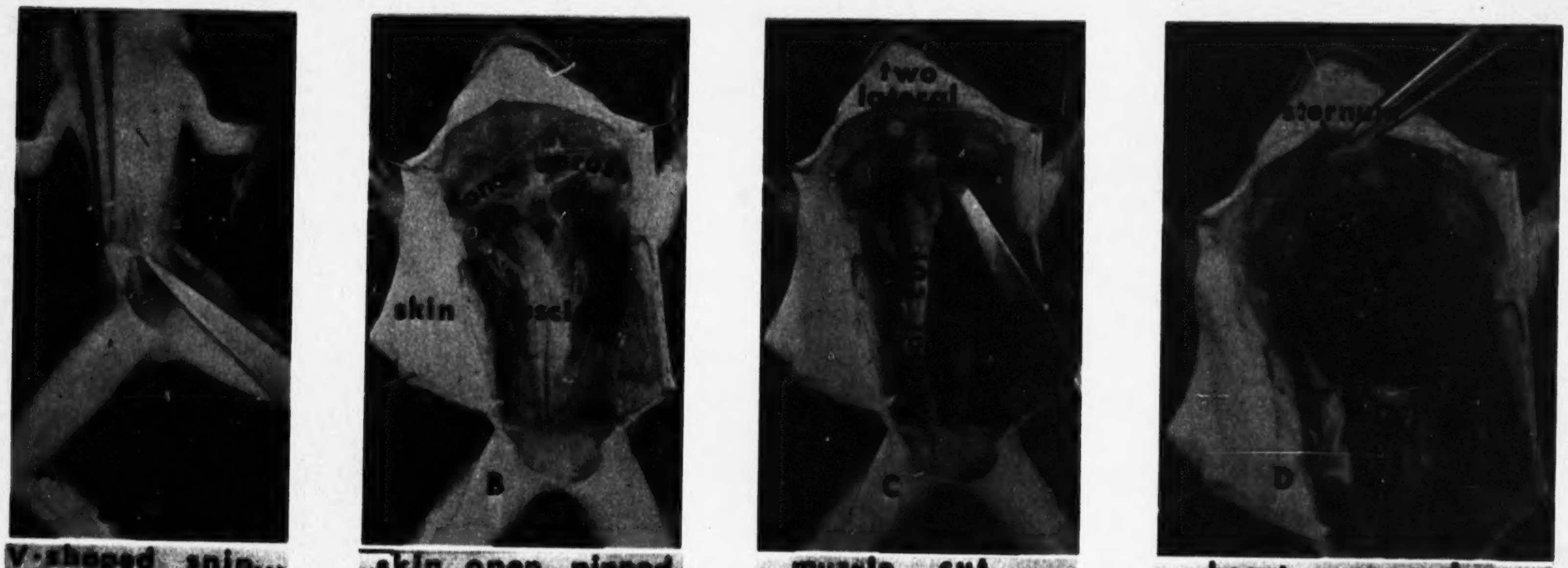
2. With forceps, lift the skin of the posterior postion of the abdomen near the bases of the hind-limbs. *See Fig. 1.20A.*

3. Make a small V-shaped snip with the scissors, then place the point of the scissors into the opening thus made and cut just the skin forward and backward as far as you can go on the ventral side. *See Fig. 1.20B.*

4. Lay the skin back and pin it to the paraffin. This exposes a thin layer of muscles covering the abdomen.

5. Repeat the above procedure (skin) on this exposed muscle covering, cutting just the muscle. When you come to the bones that extend across the ventral side of the chest, cut through them on each side of the midline and lift out the middle. Be very careful in this region: the heart lies just dorsal to it. *See Fig. 1.20C. and D.*

Fig. 1.14 *Ventral dissection of Rana pipiens.*



V-shaped snip... ..skin open, pinned. ...muscle cut... ...heart exposed.

6. Make lateral cuts in the region of the pelvis and turn out the flaps of muscle. Pin the muscle to the paraffin.

7. Subsequent dissection is best made under water, in order to support the internal organs in their most natural position.

C. OBSERVATIONS TO BE MADE.

1. You have exposed the heart when you took out the sternum (breast bone). It is surrounded by a transparent membrane, the pericardial sac. Note the regular sequence of contractions of the organ: first

the two atria (anterior) and then the single ventricle. How do you interpret the regular darkening and lightening of the heart chambers as the contractions continue? Time the contractions of the ventricle now, and at regular intervals for as long as you continue dissection. Graph this information, using time as the horizontal axis and number of contractions per minute as the vertical axis.

2. The liver is about the same color as the heart, and lies near the heart. Describe its location with reference to the heart, using the terms in part A. Lift up the posterior edge of the middle lobe of the liver and find the gall bladder, a small spherical greenish sac lying between the lobes of the liver.

3. The lungs are two thin-walled elastic sacs at the sides of the heart in the dorsal part of the body cavity. They are usually covered by the liver. The lungs are probably already inflated, but if not you should inflate them by placing a dropping pipet between the lips of the glottis at the back of the mouth, and squeezing the bulb. Observe the appearance of the lungs carefully. (It is interesting to observe these under the binocular dissecting microscope.)

4. The stomach is a white long cylindrical tube on the left side of the body cavity. (Note that the terms "right" and "left" always refer to the frog's right and left, no matter where you happen to be.) Food enters the frog's mouth, then down the slender tubular esophagus to reach the stomach.

5. Trace the intestine from the stomach, remembering that it is a single, continuous tube even if it is very much coiled. Note the double layer of mesentery which suspends all the digestive organs from the dorsal body wall. Find the long small intestine and the short large intestine. What do the terms "small" and "large" refer to in this case? The large intestine empties into the cloaca, which is very far back in the body cavity and can be distinguished from the intestine by its thicker walls and smaller diameter. The word "cloaca" means sewer. The frog's cloaca collects digestive wastes, urinary wastes, and reproductive products before they leave the body through the anus or cloacal pore. Would you expect a human to have a cloaca?

6. The pancreas is difficult to find. It is a pinkish-white irregular mass, usually very long and slender, lying between the loop made by the stomach and the first part of the small intestine.

7. Female specimen: The ovaries are variable in size, depending on the season, but at this time of year are made up to two large masses of eggs, one on each side of the body. Each of the masses is one ovary. The eggs seem to be loose and without covering, but still they are attached quite firmly to the tissue of the ovary. The oviducts, two in number, lie dorso-lateral to the ovaries, and are conspicuous white coiled tubes. Trace an oviduct forward and backward, finding each end.

8. Male specimen: The testes are a pair of yellow oval bodies attached by a mesentery to the dorsal body wall. Each has a group of yellowish finger-shaped fat bodies attached to it. (Males in this species of frog usually have a vestigial oviduct, in the same position as the oviduct in the female, but it has no function.)

9. The spleen is the color of the heart and liver; small and spherical; lying in the mesentery near the anterior end of the small intestine.

10. The urinary bladder is a thin-walled membranous sac, and lies ventral to the large intestine at the posterior end of the body cavity. Usually the dissection loosens it from its anterior attachemnt, and it slips posteriorly into a small mass. Stretch it forward, to check its elasticity. Its only duct opens into the ventral wall of the cloaca. It has no direct connection to the kidneys.

11. The kidneys are two flat, elongated, oval bodies, about the color of the liver, attached to the dorsal body wall just posterior to the ovaries or testes. How would you interpret the fact that the heart, liver, spleen and kidneys have about the same color?

When you have completed your dissection, observation and recording of data, give the frog to the assistant who will put it into 10% formaldehyde and save it for muscle dissection later in the course.

LABORATORY WEEK 2:
BIOLOGICAL CHEMISTRY

As biologists have found out more about the chemical composition of living things, and about the chemical processes that go on within them, it has become ever more important that they be able to use chemical techniques easily. Students who choose a biology major will be required to take several courses in Chemistry, but the learning of biological chemistry cannot wait for those courses. We must teach some elementary chemical techniques at once. The tests and procedures in this week's work are not difficult, either to perform or to understand. Most of them depend on the formation of a typical insoluble product (called a "precipitate"), or the production of some definite color, or both.

In the following work, enough procedure is described to get you started in the right direction, but then the procedure is up to you. Keep careful notes in your data book of the results that you get. It is not necessary to copy exact procedure from the manual into your data book, but it is necessary to make note of any deviations you made from the directions, and it is of course necessary to make careful note of the results of all tests. Work alone if you wish, or with one or two other people.

It is not enough to see that tests work on the substances they are supposed to work on--you must also see that they do not work on others. You should therefore set up your procedure to include controls: distilled water, and as many of the stock materials as you think are necessary to give definitive results. As you work, fill out the table on page 2-2, or make a similar one in your data book. This is important both for understanding and for help in determining the constitution of the food sample,

Solutions: You will find a pair of pans at each end of each laboratory table; one containing clear-colored dropping bottles and the other containing brown-colored dropping bottles. The clear ones contain the solutions to be tested; the brown ones contain the reagents with which you make the tests.

Fig. 2.1 Solutions to be tested are in clear bottles, reagents are in brown bottles. Two complete sets for each laboratory table, not to be moved from place to place.

You must not sort these around from pan to pan or from table to table--each set is complete. If any bottle is empty, you may re-fill it from the stock bottle on the side table.

TEST SOLUTION			Molisch Test	Benedict Test	Lugol's I_2KI Test	Paper Test	CCl_4 Test	Sudan IV Test	Coagulation Test	Biuret Test	Ninhydrin Test	Albustix Test	Oxalic Acid	Silver Nitrate	Barium Chloride	Ferrocyanide	Hydrochloric Acid
Distilled Water																	
Carbohydrates	Monosaccharides	Glucose															
		Fructose															
	Disaccharides	Sucrose															
		Maltose															
	Polysaccharides	Glycogen															
		Starch															
		Methyl cellulose															
Lipids		Corn oil															
		Suet															
		Nut															
		Milk															
Proteins and their derivatives	Amino Acids	Methionine															
		Glutamic acid															
	Proteins	Peptone															
		Egg albumin															
		Gelatin															
Mineral Ions		Ca^{++}															
		Cl^{--}															
		$(SO_4)^{--}$															
		Cu^{++}															
		Fe^{+++}															
		$(CO_3)^{--}$															
		$(HCO_3)^{-}$															

Description of procedures:

I. TEST FOR CARBOHYDRATES.

A. MOLISCH TEST FOR THE PRESENCE OF CARBOHYDRATES.

First observe the colors produced by the positive test with glucose and the negative test with distilled water. Put 1 ml. of glucose solution into one test tube, and 1 ml. of distilled water into another; add 2 drops of alcoholic α-naphthol to each tube and mix thoroughly. Slant the tube and carefully slide 1 ml. of concentrated H_2SO_4 down the inside of the tube, so as to form a layer beneath the stock solution. After a short time, make careful note of the reddish color at the interface. This color is the result of condensation of sugar with the α-naphthol. Next, test all the carbohydrates, comparing the strength of the colored product and the time it takes to produce it.

B. BENEDICT TEST FOR REDUCING SUGARS. Some sugars contain portions of the molecule that can be oxidized easily, whole others do not (note that "oxidized" here means loss of an electron). If they are easily oxidized, then, in the presence of a cupric Cu^{++} ion, they will give up that electron to the Cu^{++} ion and make it a colored precipitate, Cu_2O cuprous oxide which you can see. Benedict's solution contains buffered Cu^{++} ions, which become reduced by some sugars. These are called reducing sugars.

Compare the results obtained by testing glucose and distilled water as follows. Put 1 ml. of glucose solution into a test tube and 1 ml. of distilled water into another tube. Add 5 drops of Benedict's solution, and put the tubes into a boiling water bath for a few minutes. Note the color of the precipitate. Then test the other carbohydrate stock solutions in the same way.

C. IODINE TEST FOR SOME POLYSACCHARIDES.

Test 1 ml. each of distilled water, glucose, glycogen and starch with a drop of Lugol's I_2KI solution, and carefully note the color produced in each case. The reddish color of the glycogen and the blue color or the starch are both positive tests, but the yellow-brown color of the iodine alone is a negative test. Test the other carbohydrates, noting especially the peculiar reaction of the iodine in the cellulose suspension, which is nevertheless a negative reaction.

D. With a sharp scalpel or razor blade, cut an extremely thin slice of potato and mount in a few drops of water on a microscope slide. Cover and examine microscopically. Then add a drop of Lugol's I_2KI solution at the edge of the coverglass, allow it to diffuse through the potato slice, and examine again. Record in your data book, not neglecting to estimate sizes of cells and starch granules.

II. TEST FOR LIPIDS.

There is no good easy positive test for the presence of lipids as there is for carbohydrates (Molisch test). However, it is possible to test generally for the presence of lipids by using A, B or C below.

A. PAPER TEST.

Rub a bit of corn oil onto a square of tissue paper, and a bit of suet onto another. Let them dry completely, then hold the paper to the light, and note the translucence of the paper where you have rubbed the lipid. Try this with carbohydrates and protein solutions. This test is adequate for fairly concentrated lipids, but if they are diffused in suspension, you will need to concentrate them as in B below.

B. DISSOLVING IN CCl_4

First, rub a drop of CCl_4 onto a square of tissue paper, let it dry, hold the paper to the light, and see that CCl_4 alone does not turn the paper permanently translucent. Then mix 1 ml. of CCl_4 and one drop of corn oil in a test tube, rub some of this solution onto a square of paper, let dry, and observe the translucent spot. Now take 2 ml. of homogenized milk from the refrigerator, add 1 ml. of CCl_4 to it, and shake thoroughly to force the CCl_4 to dissolve the suspended butterfat droplets. Let the two phases separate, with the watery milk residue above and the CCl_4 below. Test each phase with paper, allowing them to dry thoroughly.

C. SUDAN IV DYE TEST.

You cannot use this test in conjunction with CCl_4, because although Sudan IV is insoluble in water, it is highly soluble in CCl_4. This test checks to see if there is lipid present in a watery mixture -- if any of the dry dye dissolves, there is lipid present; provided you have not added fat solvents such as CCl_4 or alcohol. First test the solubility of a few crystals of Sudan IV in water, by dropping them with forceps onto the surface of 5 ml. of water in a test tube and shaking hard. Now add 1 ml. of corn oil to the water, shake, and see that the dye dissolves only in the oil.

D. SKIM MILK. Using the tests above, determine whether or not you can detect the presence of butterfat in skim milk.

E. MICROSCOPIC EXAMINATION. One of the above tests will probably suffice to detect lipids in foodstuffs, etc., but biologists usually want to know where the lipid is in living material. For this, one must stain the lipid with some lipid-specific dye, and then examine with a microscope.

1. Make a very thin slice of an avocado or a pecan, mount the slice in water on a microscope slide, add a coverglass, and examine with low power of a compound microscope. Remove the coverglass, add a few crystals of Sudan IV, replace the coverglass, and after a few minutes examine again. Can you see where the lipid is located?

2. Permanent slides can be made, using silver nitrate or osmic acid, both of which turn black or brown in the presence of lipids. Observe the demonstration slides on the window table: spinal cord stained with silver nitrate, and adipose (fat) tissue stained with osmic acid. In nervous tissue, most of the lipid is concentrated in the sheaths around nerve fibers; while in adipose tissue it is in the fat droplets inside the fat cells. Sketch these preparations as seen under high power, recording the sizes of the objects.

III. TESTS FOR PROTEINS AND PROTEIN DERIVATIVES.

Note: the egg white suspension and the gelatin are whole proteins; the peptone is a protein that has been broken down into smaller chains of amino acids; and methionine and glutamic acid are purified amino acids. Methionine is an amino acid that contains sulfur in its structure. Glutamic acid used in the laboratory is actually a salt of that amino acid, mono-sodium glutamate ("Accent").

A. COAGULATION TESTS. Some proteins tend to become coagulated by harsh conditions such as heat, metal salts, acids and alkalis. Test about 1 ml. each of the proteins and derivatives by boiling them, and record which of them produce a precipitate..

B. BIURET TEST. Test 1 ml. of a protein or protein derivative as follows: Add and mix 10 drops of concentrated NaOH, and a few drops of 0.5% $CuSO_4$. Observe the color very carefully. A positive test is reddish, but a blue color is not a positive test. (This test should be positive for materials containing peptide linkages.)

C. NINHYDRIN TEST. Test 1 ml. of a protein or protein derivative as follows: Add and mix 1 ml. of ninhydrin reagent, place in a boiling water bath and leave there until you observe a bluish, purplish, or pinkish color. This may take as long as 20 minutes, if the protein is very dilute. (This test should be positive for materials containing free amino groups.)

D. ALBUSTIX TEST. This test is primarily used to detect protein in urine. Put a drop of egg white solution on the colored end of an albustix and observe the color. Repeat with other proteins and derivatives.

IV. TESTS FOR MINERAL IONS.

A few mineral ions that are found abundantly in living things are easily detected by simple tests such as the following examples. Other ions are often more difficult to detect.

A. CALCIUM ION, Ca^{++}

To 1 ml. of $CaCl_2$ stock solution add a few drops of 5% $H_2C_2O_4$, and observe the calcium oxalate precipitate. (A salt of oxalic acid, sodium oxalate, is used to remove calcium ions from blood to prevent its clotting.)

B. CHLORIDE ION, Cl^-.

To 1 ml. of $CaCl_2$ stock solution add a few drops of 2% $AgNO_3$ and observe the silver chloride precipitate, AgCl.

C. SULFATE ION, $(SO_4)^{--}$.

To 1 ml. of $CuSO_4$ stock solution add a few drops of $BaCl_2$ and observe the $BaSO_4$ precipitate, barium sulfate.

D. COPPER ION, Cu^{++}.

To 1 ml. of $CuSO_4$ stock solution add a few drops of $K_4Fe(CN)_6$ solution and observe the $Cu_2Fe(CN)_6$ precipitate, copper ferrocyanide.

E. IRON ION, Fe^{+++}.

To 1 ml. of $FeCl_3$ test solution add a few drops of $K_4Fe(CN)_6$ solution and observe the precipitate, ferric ferrocyanide.

F. CARBONATE ION, $(CO_3)^{--}$ and bicarbonate ion, $(HCO_3)^-$.

To 1 ml. each of Na_2CO_3 and $NaHCO_3$ stock solutions, add a few drops of 2% HCl, and observe the CO_2 carbon dioxide gas being produced in bubbles.

V. ANALYSIS OF A FOOD SAMPLE

Each person should do the following as an independent project, and each person should write his own report on the project.

Bring a fresh sample of some food to the laboratory, run it in the blendor if necessary, and determine what carbohydrates, lipids, proteins and mineral ions it contains.

VI. REPORT #1.

Refer to the Introduction of this manual for the proper form for a scientific report, and to page 2 - 11 for an example. The topic for discussion in your Conference this week will be the proper reporting of scientific information, so it will be well if you read that part of the Introduction beforehand. You might also try writing the report before the Conference, but do not put it into finished form until after the group has discussed the subject.

VII. NUCLEIC ACIDS.

We do not have an easy method for testing for nucleic acids such as we have for I - IV above. There are excellent methods available, but they require more equipment than we have at our disposal. Therefore we will look only at a method of extracting a mixture of DNA and RNA, to see generally what these nucleic acids look like. Do not attempt to test other stock materials or unknown samples for nucleic acids -- the results are not worth the effort. The following procedure can be used with any fresh materials that can be ground finely, such as meat, liver, etc. The formation of fibers signifies the presence of nucleic acids.

A. EXTRACTION AND EXAMINATION

Put 5 ml. of dry powdered wheat seeds into a beaker, and add 10 ml. of a 2% solution of sodium lauryl sulfate (a detergent). Stir occasionally for one-half hour or more, until the mixture becomes less viscous (sticky) than it was to start. Centrifuge the mixture for about ten minutes. Check with the assistant if you do not know how to use a centrifuge, and make sure that you leave the centrifuge tubes clean when you have finished.

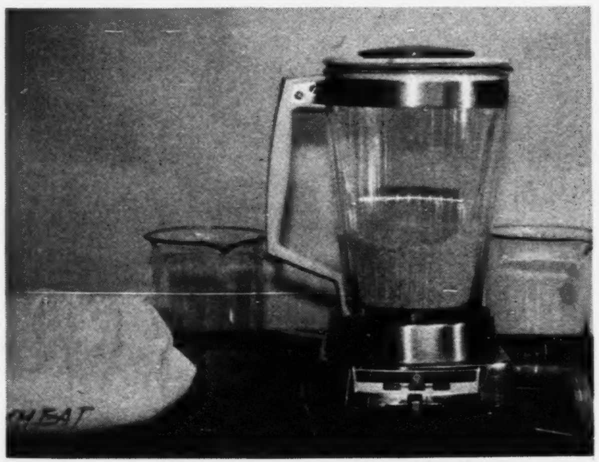


Fig. 2.2 Wheat powdered dry in the blendor. Add detergent, stir.

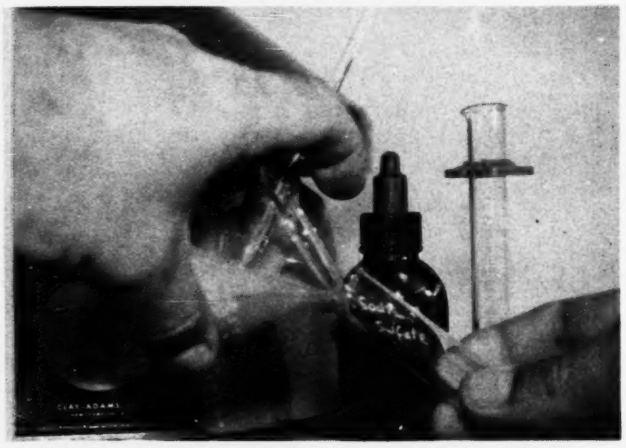


Fig. 2.3 Pour liquid into centrifuge tube, and discard solid remainder.

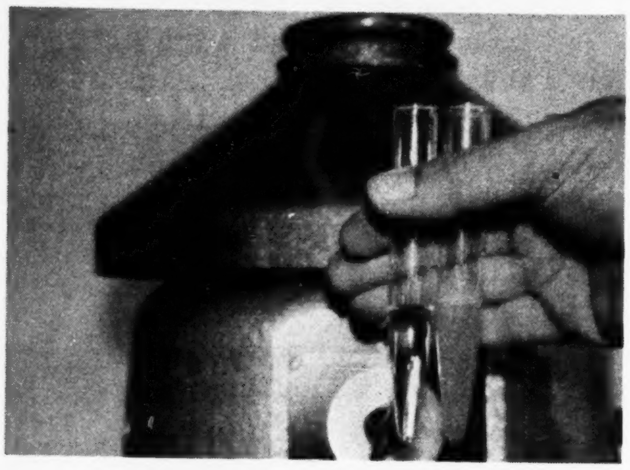


Fig. 2.4 Put an exactly equal volume of water into another centrifuge tube, to act as a balance in centrifuge.

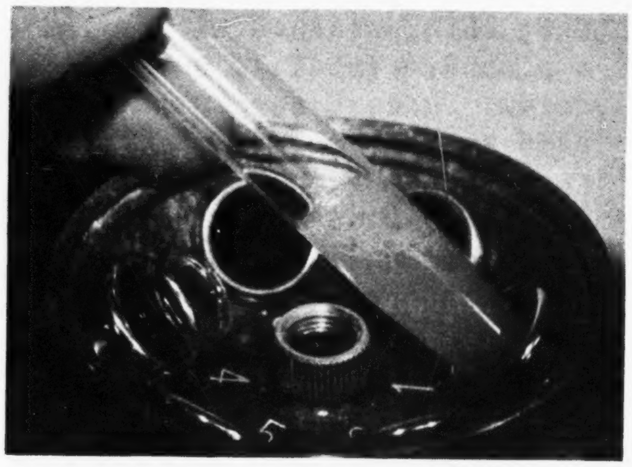


Fig. 2.5 Lower tubes gently into opposite holders. Cap centrifuge, run at speed 4, 10 minutes, allow to self-stop.

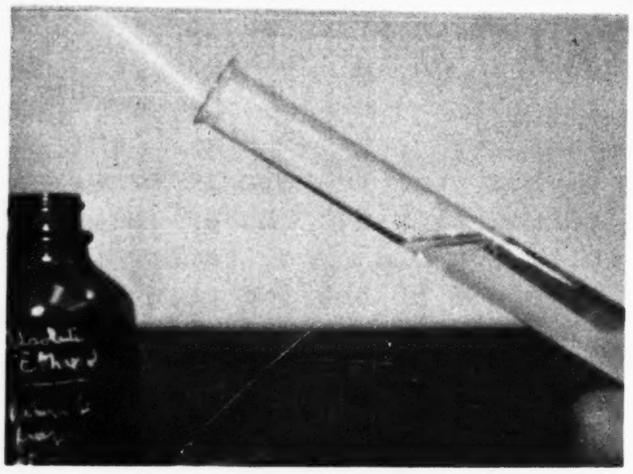


Fig. 2.6 Pour clear supernatent into a test tube, add an upper layer of very cold absolute ethanol.

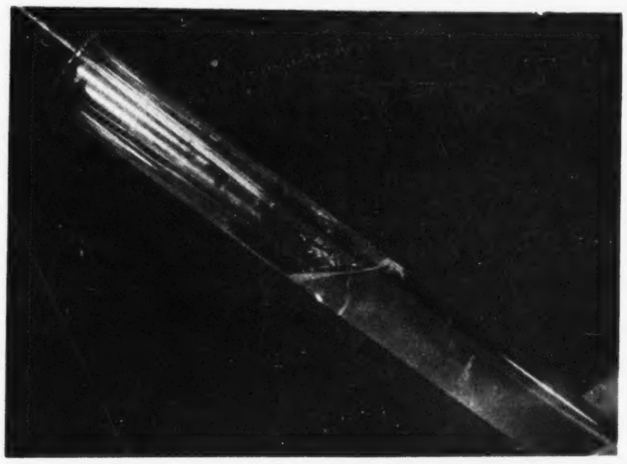


Fig. 2.7 Put hooked glass rod through the ethanol, then pull it up into the ethanol. Fine nucleic acid threads should form.

Pour about 5 ml. of the clear upper solution, which contains the nucleic acids, into a test tube, and then slide about 5 ml. of very cold ethanol down the side of the tube to make an ethanol phase above the nucleic acid mixture. Put a hooked glass rod down through the ethanol into the nucleic acid phase, then pull it back up through the ethanol. There should be a fine fiber formed on the rod. Repeat this procedure several times, and spread the fibers out in the centers of two microslides. Keep the fibers wet with ethanol, cover with a coverglass, and examine microscopically with high power. Remove the coverglass and allow the slides to dry in air. Label and save the slides for possible later use.

B. MICROSCOPIC PREPARATIONS. We now know that most of the nuclear stains used in biological preparations are attracted to the nucleic acids in the nucleus. Examine the following slides:

1. Hematoxylin stain of sloughed frog skin. Hematoxylin, or purified logwood, is a purple-colored stain attracted generally to nucleo-

protein mixtures. It is probably the most widely-used stain in biology. The slide on demonstration (window table) is of whole mounts of thin frog skin, stained with Delafield's hematoxylin and counterstained with a pink dye (eosin), which is attracted to protein rather than to nucleic acids. Record carefully where you find each dye. The counterstain is used so you can see the rest of the cell, not just the nucleoproteins.

2. Heidenhain's hematoxylin stain of chromosomes in onion root tip. This hematoxylin is treated differently and concentrates so deeply in the nucleoprotein that it looks black. No counterstain is used here. Where is the nucleic acid? Find cells in division, with the chromosomes readily apparent. We will study these slides in greater detail later in the course.

3. Feulgen's stain for DNA in onion root tip. This stain colors only the DNA, and not the RNA at all. A greenish counterstain is used, so you can see the rest of the cells. Where is DNA located?

4. Azure B stain, to differentiate between DNA and RNA. There are two colors present in this slide--bluegreen and purple. Which of these is probably DNA? Which is RNA? Why do you think so?

VIII. MEASUREMENT OF GASES WITH A MANOMETER.

When a living animal breathes, it removes some of the oxygen from the air it breathes and replaces it with an approximately equal volume of carbon dioxide. Later in the course we will study the complex processes that lead to the combining of carbon with oxygen to make the carbon dioxide, but we may say now that one of the important processes of animals is this breathing process. The formula for the oxidation of a monosaccharide sugar will suffice for the present:

$$C_6H_{12}O_6 + \boxed{6O_2} \longrightarrow \text{many intermediate processes} \longrightarrow \boxed{6CO_2} + 6H_2O$$

In this case, the number of oxygen and carbon dioxide molecules are equal, though this is not true in the oxidation of all foods. An important fact to remember is that these equal number of molecules of different gases will occupy the same volume, if they are kept at the same temperature and pressure.

If, then, we can enclose a breathing animal in an atmosphere without CO_2, and remove CO_2 from the air it breathes out, the oxygen in the air should gradually be reduced in volume; and the pressure in the chamber should become reduced because there is no CO_2 available to take up the space. The measurement of this change in pressure is the basis for all study with manometers.

First we must have a means of removing CO_2 from the air.

A. FORMING A PRECIPITATE TO TIE UP CARBON DIOXIDE.

When carbon dioxide is bubbled through water, most of it will become dissolved in the water and some of it will react with the water like this:

$CO_2 + H_2O \longrightarrow H_2CO_3$, carbonic acid.

This is the form in which your blood carries most of its carbon dioxide--that is, as carbonates and bicarbonates. Carbonic acid will react with almost any hydroxide to form a salt, and if the salt is insoluble, it will precipitate from the solution. One of the most insoluble of these is $Ba(OH)_2$.

$$Ba(OH)_2 + H_2CO_3 \longrightarrow BaCO_3\downarrow + 2H_2O$$

Filter 5 ml. of saturated $Ba(OH)_2$ into a test tube, making sure that the solution is absolutely clear. Bubble your breath gently through it, using a drinking straw, and note the rapidly-forming $BaCO_3$ precipitate. With another sample of $Ba(OH)_2$ simply swirl the clear sample around to combine it with air.

NaOH and KOH, sodium hydroxide and potassium hydroxide, will form carbonates with CO_2 also, and even more readily, but their carbonates are more soluble in water and will not appear as rapidly as precipitates. Demonstrate this by bubbling your breath through these solutions. Caution: these solutions can burn, and you must not get them in your eyes! How could you tell if Na_2CO_3 or K_2CO_3 were present? (See IV, F above.)

B. MEASURING OXYGEN UPTAKE.

Set up a manometer according to the photographs below. Saturate filter paper with KOH just before closing the top of the bottle. Leave the clamp off until the entire system has come to equilibrium, then close. Any variation in temperature will make your results invalid, because of expansion or contraction of the air in the bottle, and therefore the manometer bottle must be kept in a constant-temperature water bath. Run at room temperature; then by immersing the bottle in colder or warmer water, run the experiment a few degrees above and a few degrees below room temperature.

Compute the volume change in the bottle, in cubic millimeters (mm^3).

Graph all three sets of data on one graph for comparison purposes; using minutes of time as the horizontal axis and mm^3 of oxygen used as the vertical axis.

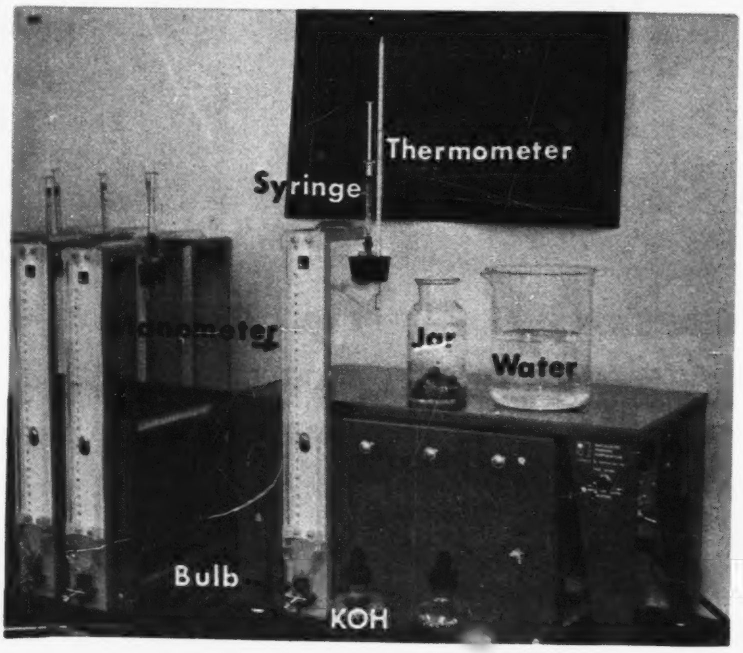


Fig. 2.8 Components of manometer.

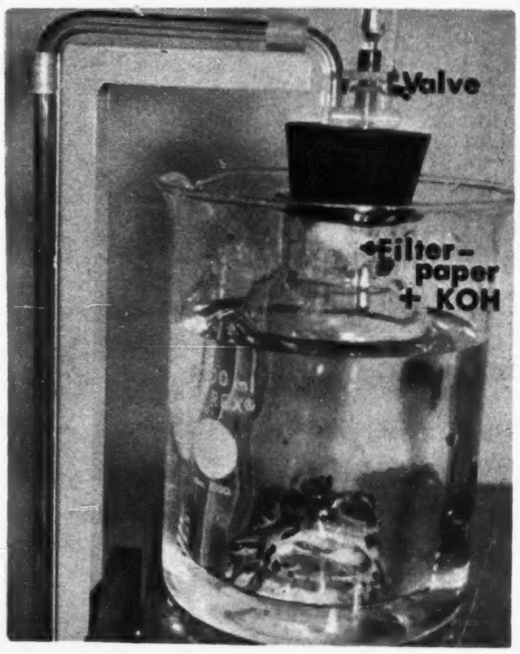


Fig. 2.9 Manometer components assembled.

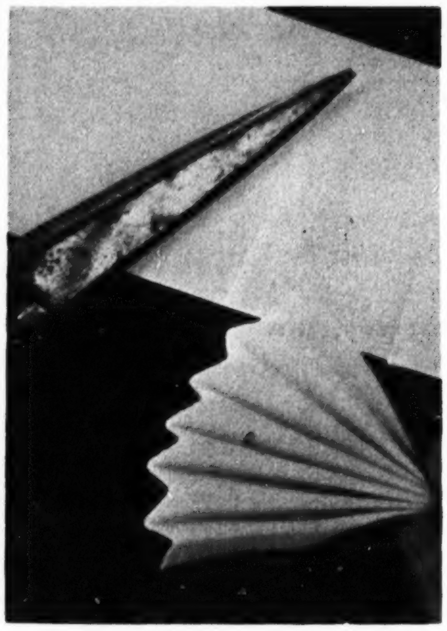


Fig. 2.10 Fold filter-paper strip into fan shape.

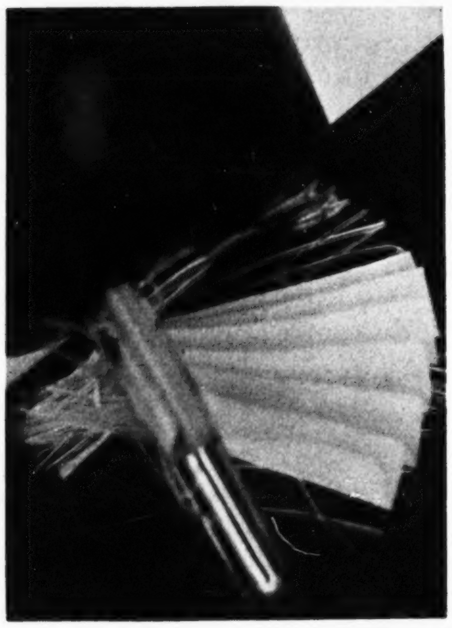


Fig. 2.11 Insert paper into holder; add fresh KOH just before stoppering jar.

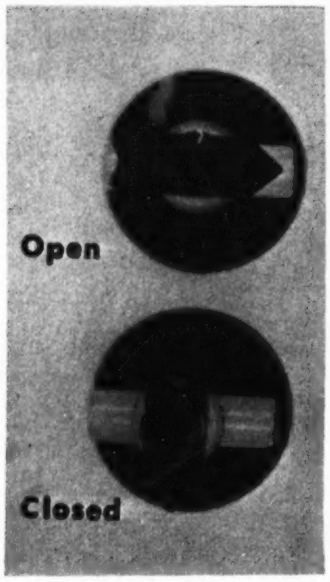


Fig. 2.12 Keep valve open until temperature is stable. Put syringe at zero.

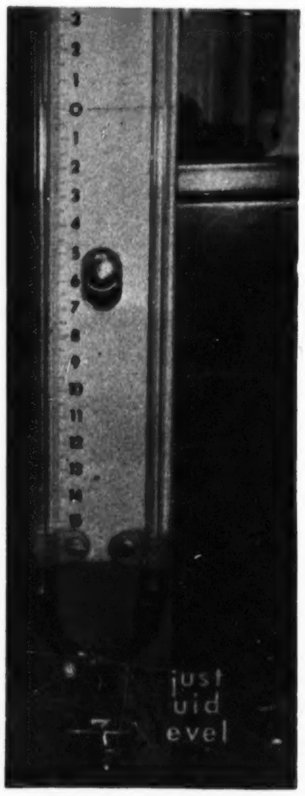


Fig. 2.13 Adjust manometer fluid in tubes to zero, then close valve and check time.

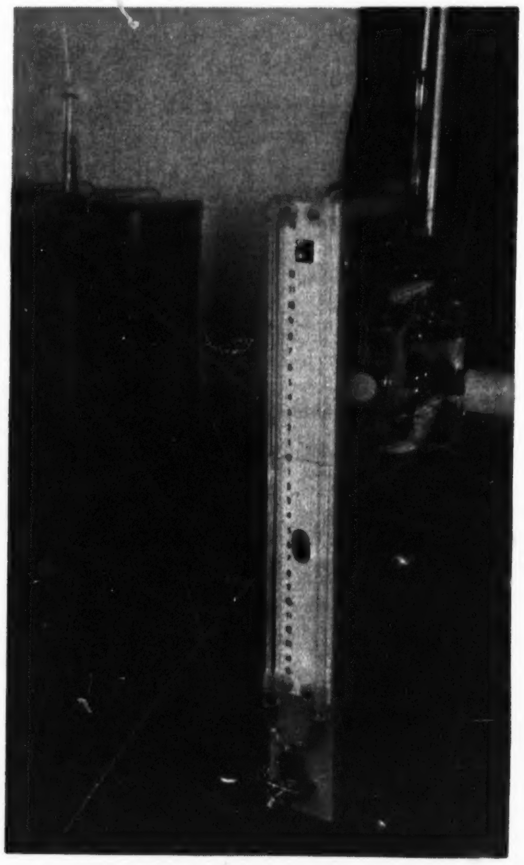


Fig. 2.14 Fluid should lower in left-hand tube. Why? If it does not, the system leaks. Run long enough for accurate results.

Fig. 2.15. Return manometer fluid to zero by depressing syringe. Read volume of O_2 used on syringe.

IX. A SAMPLE REPORT. The following is a sample writeup of an imaginary analysis, presented as a technical help in your writing.

ANALYSIS OF SOME COMPONENTS OF "BAKED ALASKA"

Introduction.

In response to the instructions on page 2 - 6 of (1), this investigator studied the carbohydrates, lipids, mineral ions, proteins and protein derivatives in a sample of Baked Alaska. According to the recipe on page 355 of (2), this dessert consists of ice cream covered with meringue, placed on a board and baked in a hot oven. The cook book (2) contained a recipe for the meringue, but the investigator was not sure which of several possible recipes were followed in making the ice cream.

Since the Baked Alaska portion melted and separated during its transporation, it was decided to analyze the two fractions separately as far as possible. After checking page 190 of (3), it was believed that the meringue fraction would contain protein and sugar; and the ice cream fraction would contain sugar, lipid, and protein. No hypothesis was made concerning the content of mineral ions, since (3) listed very small percentages of them and the investigator was not sure how much of the chemical was needed to form a precipitate.

Materials and Methods.

The sample of Baked Alaska, approximately 20 grams, was obtained at Charlie's Cafe Exceptionale, Minneapolis, at 9:00 p.m. on October 25, 1968, and was carried to the laboratory in a foil "Bowser Bag" after having spent overnight on a windowsill in Sorin Hall at approximately 22°C. Analysis was performed from 8:00 to 10:00 a.m. on October 26, 1968. All that would pour out of the bag was placed in a clean 100 ml. beaker, and was termed the "ice cream fraction". The remainder was scraped out, placed in a blendor, diluted with 20 ml. of distilled water, the blendor was run at high speed for three minutes, and was termed the "meringue fraction".

Tests were made as described on pages 2 - 3, to 2 - 5 of (1), except in all cases the meringue fraction and the ice cream fraction were substituted for the known samples. Attempts were made to centrifuge the samples to minimize cloudiness and floating particles, but they would not settle.

Observations.

In its original condition, the Baked Alaska was sweet, cold, and smelled like vanilla. The following results were obtained in the laboratory:

TEST:	ICE CREAM FRACTION:	MERINGUE FRACTION:
Molisch	Red at interface	Pink at interface
Benedict	Yellow-orange precipitate	No precipitate
Lugol's I_2KI	Yellow-brown, no blue	Yellow-brown, no blue
Paper	Translucent when dry	Opaque when dry
CCl_4	(not performed)	Paper faintly translucent
Sudan IV	(not performed)	No dye dissolved
Coagulation	No precipitate apparent	No precipitate apparent
Biuret	Possible faint reddish color	Reddish color
Ninhydrin	Very pale pinkish-purple	Bluish-purple
Oxalic acid	Possible white precipitate	White precipitate
Silver nitrate	No precipitate apparent	White Precipitate
Barium chloride	No precipitate apparent	White precipitate
Ferrocyanide	No precipitate apparent	No precipitate
Hydrochloric acid	No bubbles	No bubbles

The observations of the above tests were complicated by the very cloudy appearance of the ice cream fraction.

Interpretation.

Since both fractions showed a positive Molisch test, both probably contained some carbohydrate. The positive Benedict test with the ice cream fraction, and the negative Benedict test on the meringue fraction, plus the negative Lugol's I_2KI test on both, would lead one to believe that the carbohydrate was not starch or glycogen in either fraction, but that the carbohydrates were two or more different kinds of sugar. The investigator would guess that the sugar in the ice cream fraction contained some glucose, fructose or maltose, while the sugar in the meringue fraction was primarily sucrose.

The ice cream fraction contained a large enough proportion of lipid to make the paper translucent directly, making further tests unnecessary, but even when the lipid was extracted from the meringue fraction with CCl_4 the results were questionable. The fact that no Sudan IV dissolved in the meringue fraction would lead one to believe that there is little or no lipid in that fraction.

Neither fraction coagulated with heat, which was surprising since the recipe calls for egg white. However, the recipe also says to bake the egg white, and therefore the white precipitate in the meringue fraction could have been the egg white that had already been coagulated. A comparison of the results of protein testing between the two fractions indicates that the meringue fraction contained more protein than the ice cream fraction, even when highly diluted. Apparently the proteins in both fractions contained both peptide bonds and free amino groups, which is reasonable if one checks the formulas for proteins on pages 122 - 124 of (4).

No interpretation is possible concerning the mineral ions in the cloudy ice cream fraction, but it appears that the meringue fraction contained calcium, chloride and sulfate ions. The salt added to the meringue could account for the chloride; egg white contains sulfur groups which could account for the sulfate; but it is impossible to interpret the calcium ions without further knowledge.

Discussion.

If this experiment were repeated, it might be a good plan to use a large amount of CCl_4 on the ice cream fraction, thus dissolving out the lipid and perhaps leaving a clearer solution for analysis.

The investigator affirmed, under the conditions of these experiments, that the ice cream fraction contained sugar, lipid and protein, and that the meringue fraction contained protein and sugar. One surprise was the discovery that the meringue fraction also contained several mineral ions.

References.

(1) Downing, William L., Open Laboratory 1, edition 2, revised, 1968.

(2) Rombauer, Irma S., The Joy of Cooking, edition 2, 1962.

(3) Spector, William S., (editor), Handbook of Biological Data, 1956.

(4) Weisz, Paul B., The Science of Biology, edition 3, 1967.

X. VITAL STAINS.

The following is included here as a reference only, to which you can refer during future microscopic study of fresh cells and tissues, when you want to see cells or parts of cells more clearly. Use these on fresh material that is mounted in water, either by dropping one or more drops onto the cells before the coverglass is added, or by placing a drop next to the edge of the coverglass and allowing it to diffuse across.

The advantage to vital stains is that they do not ordinarily kill living cells, and you can therefore study them in a living condition. The disadvantage to them is that they are not specific in their attraction to chemical compounds, and are not therefore very useful from a histochemical point of view. They are, however, attracted somewhat differentially, as follows:

A. PROTEIN STAINS. These are attracted primarily to proteins, and are therefore most useful in staining the cytoplasm of cells. The last two are often used to stain mitochondria.

1. Brilliant vital red.
2. Trypan blue.
3. Crystal violet.
4. Janus Green B.

B. LIPID STAINS. These are attracted primarily to lipids, and are therefore most useful in staining the inner and outer membranes of cells, which are made primarily of lipid.

1. Brilliant cresyl blue.
2. Nile blue A.

C. NUCLEIC ACID STAINS. You cannot use these to differentiate between DNA and RNA, but they will be attracted to both nuclear and cytoplasmic nucleic acid.

1. Hematoxylin
2. Methylene blue
3. Neutral red
4. Safranin O

These stains will be kept permanently on the front table of the laboratory, and you should feel free to take and use them at any time.

LABORATORY WEEK 3:
MONERA

In this course, Bacteria and Bluegreens are classified together as "Monera", because they both lack nuclear membranes and in general resemble each other biochemically. This system of classification is not the most common one in use today, but using it helps us in understanding generalizations about them. A more commonly used system is to call the Bluegreens the most primitive of the Algae (green Thallophyte plants) and Bacteria members of the Fungi (non-green Thallophyte plants). The primary interest in bacteria is in the diseases they cause, and the science of Bacteriology has developed around these forms, in which the bacteriologists use some of the most sophisticated techniques to be found in Biology today. You will study a few of these techniques this week.

I. THE PECULIAR NUCLEAR ARRANGEMENT.

Examine the slides on demonstration microscopes of bacteria (Bacillus cereus) and bluegreens (Oscillatoria) stained for nucleic acids. If a distinct nucleus were present, it would be stained. Look very carefully to see where the nucleic acids are located in the cells, and make accurate records of your observations. (The microscopes used are oil immersion ones, with magnification of 970x.)

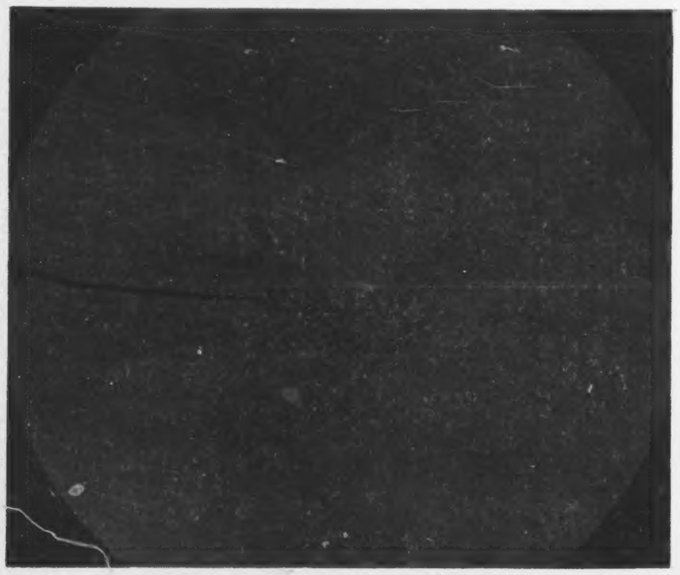


Fig. 3.1 Hundreds of Bacillus cereus cells, nuclear stain, as seen with oil immersion microscope (x970).

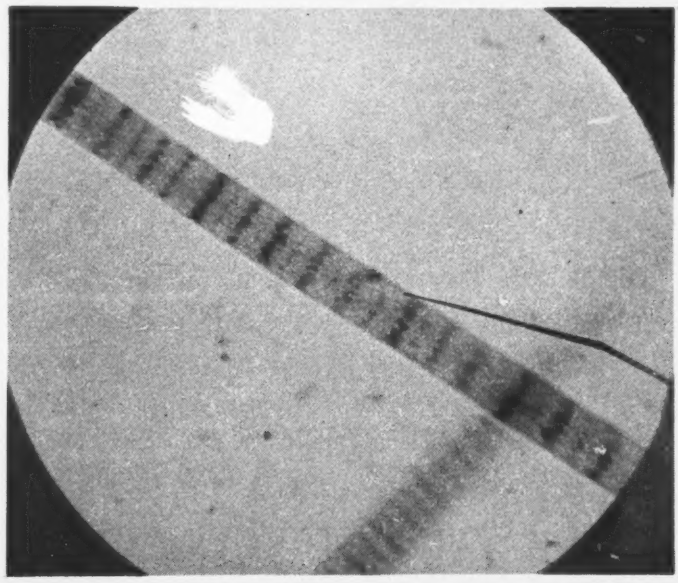


Fig. 3.2 One colony of Oscillatoria cells; nuclear stain, as seen with oil immersion microscope (x970).

II. OBSERVATIONS OF LIVING BLUEGREENS.

Under the best of conditions, it is difficult to distinguish different parts of bluegreens. Make sure that you reduce the light as with all living preparations, and that you do not try to look at took thick a mass of culture.

Cells of bluegreens may be spherical or elongated, occur singly or in groups, groups may be end-to-end or in masses, and "colonies" are more than likely surrounded by masses of gelatinous polysaccharide slime. Be sure you know how much of what you are looking at is a single cell.

A. GLEOCAPSA. The name means "glue-case." Put two or three drops of the culture material on a slide and cover with a coverglass. Under low power the cells appear to be tiny bluegreen dots. Examine under high power. The cells are pigmented, and are surrounded by several gelatinous capsules. Often several cells, with their capsules, are fastened together with still larger capsules, giving the effect of a colony.

B. OSCILLATORIA. Mount only a small amount of the filamentous material together with several drops of water, and tease the filaments apart if necessary in order that you will get the strands separated. In this bluegreen, the cells are cylindrical and are fastened together end-to-end to make a filamentous colony. Can you distinguish the separate cells? Watch a colony moving, and be able to describe it.

C. NOSTOC. Mount this in the same way you did Oscillatoria. Is this made up of one cell or of colonies? Are the individuals able to move? How does this differ from the preceding two forms?

III. MORPHOLOGY OF BACTERIA.

A. STUDY THESE SIX SPECIES OF BACTERIA:

1. Micrococcus roseus
2. Sarcina lutea
3. Bacillus subtilis
4. Escherichia coli
5. Streptococcus lactis
6. Rhodospirillum rubrum

B. STUDY EACH OF THESE SPECIES IN ALL THREE OF THE FOLLOWING WAYS:

1. As they look when grown on agar. Nutrient agar plates of these six species have been made and incubated. Notice that the colonies of bacteria grow in different ways. Be able to describe the colonies as to size, shape, edges, color and surface.

2. In the living condition under high power of the microscope. Put two or three drops from the broth culture onto a slide, add a coverglass, and examine. When they are un-stained it will be difficult to see the shape of the cells, but easier in 3 below. However, you may want to use one or more of the vital dyes available. Look especially for movement or other activity.

3. Stained with Gram stain. This is the usual stain used by bacteriologists, primarily because it is a differential stain--some bacteria stain blue and others stain pink--and so the stain can be used to tell different species apart. Make your first Gram stain using S. lactis and E. coli, mixed together on a slide. This will allow you to tell if you do the staining properly. When examined, one kind should be blue and the other kind pink. If this is not true, you did not stain correctly. Procedure:

a. Clean a slide thoroughly with ethanol to remove all traces of oil. Put a drop of water on the clean slide, touch a flamed loop to a colony of S. lactis, stir the loop in the water, re-flame the loop and repeat with E. coli. Spread the drop out thinly, and let it dry in the air. When dry, warm it (not hot) over a burner.

b. Put the slide on the rack over the sink, and add enough Gram crystal-violet stain to cover the slide completely. Let is stand two minutes.

c. Wash the stain off the slide with tap water, then add Gram iodine solution to cover the slide. Let it stand one minute.

d. Wash the iodine off the slide with tap water, Tilt the slide lengthwise, then add ethanol drop by drop until no more purple dye can be seen washing off the slide.

e. Wash off the ethanol with tap water. At this point you can pause in the procedure to make sure that one kind of cells retains the crystal violet, as follows. Allow the slide to dry in air, and examine under high power. Those cells that have been stained purple have held onto the dye firmly, and are called "Gram positive," while those that have lost the dye are called "Gram negative". These colorless cells are difficult to see, so bacteriologists usually add a step to counterstain them and make them visible:

f. Put the slide back on the rack, cover it with Gram safranin, and let it stand one minute.

g. Wash with tap water, let it dry in the air, and examine. Now the Gram negative cells are red or pink, and are easily seen. From your knowledge of bacterial cells, and of the vital stains used previously, what do you think each stain colors?

Which species in the mixture is Gram positive? Which is Gram negative? Examine all six bacterial species, on demonstration microscopes under oil immersion. Look for the shape of cells especially: be able to tell which are rod-shaped (= bacillus), which are spherical (= coccus), and which are corkscrew shaped (= spirillum). The genus names will give you a clue in some cases.

IV. SENSITIVITY TO ANTIBIOTIC AGENTS.

Antibiotics are agents that appear to interfere with the normal metabolism of bacteria by taking the place of some biochemical that is essential to the nutrition or growth of the cells. Different species of bacteria, and different strains of the same species, will have different responses to the same antibiotic. With the development of so many antibiotics, some tool was needed to check the action of an antibiotic on particular bacteria before a prescription is given to a patient. If you will assume that E. coli is a disease-producing species (instead of the rather benign form that it really is), and that it has been isolated from a patient; you can follow through the procedure below and get an idea of how the right antibiotic might be chosen.

1. Prepare a pour-plate of E. coli, distributing the bacteria evenly as follows:

    a. Melt a tube of nutrient agar in a water bath, and then cool it until it is warm, but not hot to touch.

    b. Work quickly so you pour the agar before it hardens: Dip a sterile inoculating loop in a broth culture of E. coli, then dip it into the warm agar, and mix gently but thoroughly. Re-flame the loop.

    c. Pour the mixture into a sterile plastic petri dish. Let it harden.

2. Place the sensi-disc dispenser directly over the exposed agar, and pull the lever firmly clockwise. Eight sensi-discs should drop onto the surface of the agar. You may want to press the disc down with a flamed loop before you invert the plate for growth.

3. Store, cover down, for 24 to 48 hours, and examine.

4. Assuming all other things to be equal, which antibiotic would you choose to give to the patient? What other factors might you take into consideration before making a final choice?

V. ISOLATING AND STUDYING A CULTURE OF WILD BACTERIA.

NOTICE: TREAT ALL BACTERIA WAS THOUGH THEY WERE PATHOGENIC, BECAUSE IN CULTURING WILD BACTERIA IN THIS WAY, IT IS VERY LIKELY THAT MANY OF THEM WILL BE!

A. CONTAMINATE A PETRI DISH of nutrient agar in some particular way. Choose some contaminant that you would like to know something about: a fly's feet, or saliva, or dirty hands, etc. The agar holds the bacteria in one place, and the nutrients in it are adequate for growth of most bacteria. After contamination, mark the dish clearly and place it in your desk drawer until the bacteria grow out. At ordinary room temperature, you should have good growth in from 24 to 48 hours.

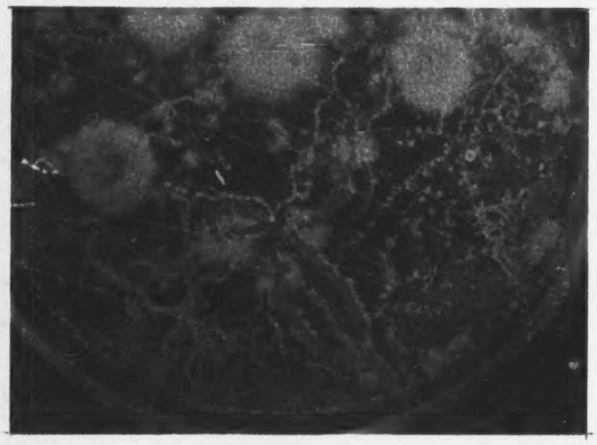


Fig. 3.3
A good mixed bacterial growth. This developed after 48 hours in a locker drawer at room temperature. The fuzzy colonies are fungi, not bacteria, and you should stay away from them and avoid blowing your spores around.

B. SEPARATE various kinds of bacteria by streaking with a loop or needle onto another petri dish of nutrient agar. This technique allows you to deal with the offspring of a single bacterium. Your objective here is to obtain colonies widely enough separated from each other so they do not grow together and contaminate each other.

1. Flame loop to red hot, all the way up to the handle. It will cool quickly in the air.

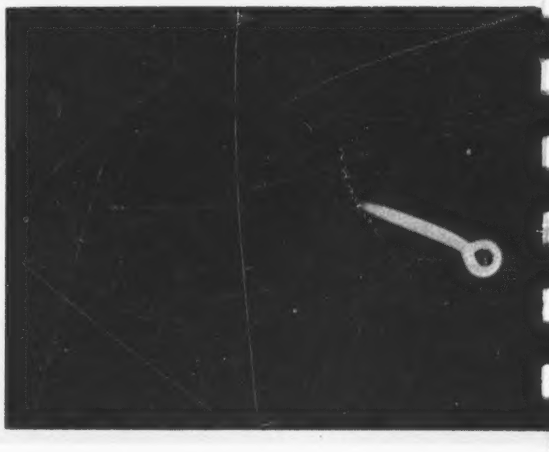


Fig. 3.4
The flamed loop.

2. Touch the flamed loop to a single isolated colony of bacteria. More than enough bacteria will stick to the loop; you need not scoop or scrape up any material.

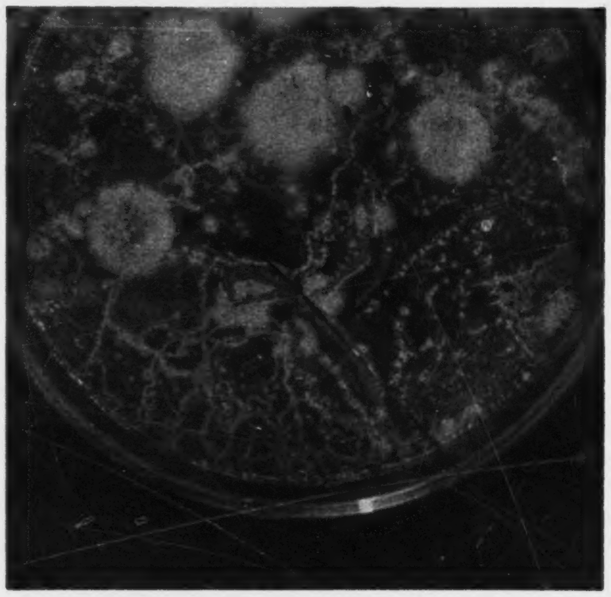


Fig. 3.5
Touch loop to a single isolated colony.

3. Take a sterile petri dish of nutrient agar and streak the loop across the agar as in the photograph below.

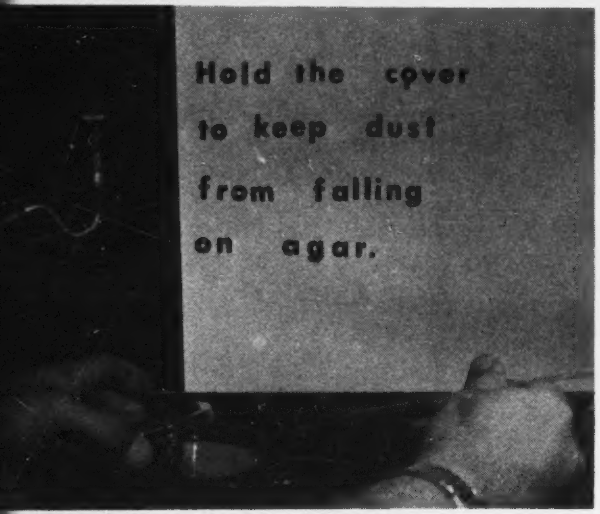
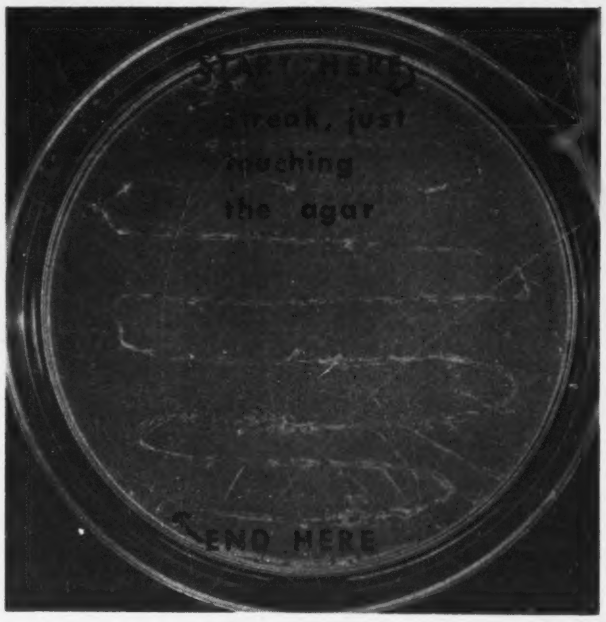


Fig. 3.6
Streaking.

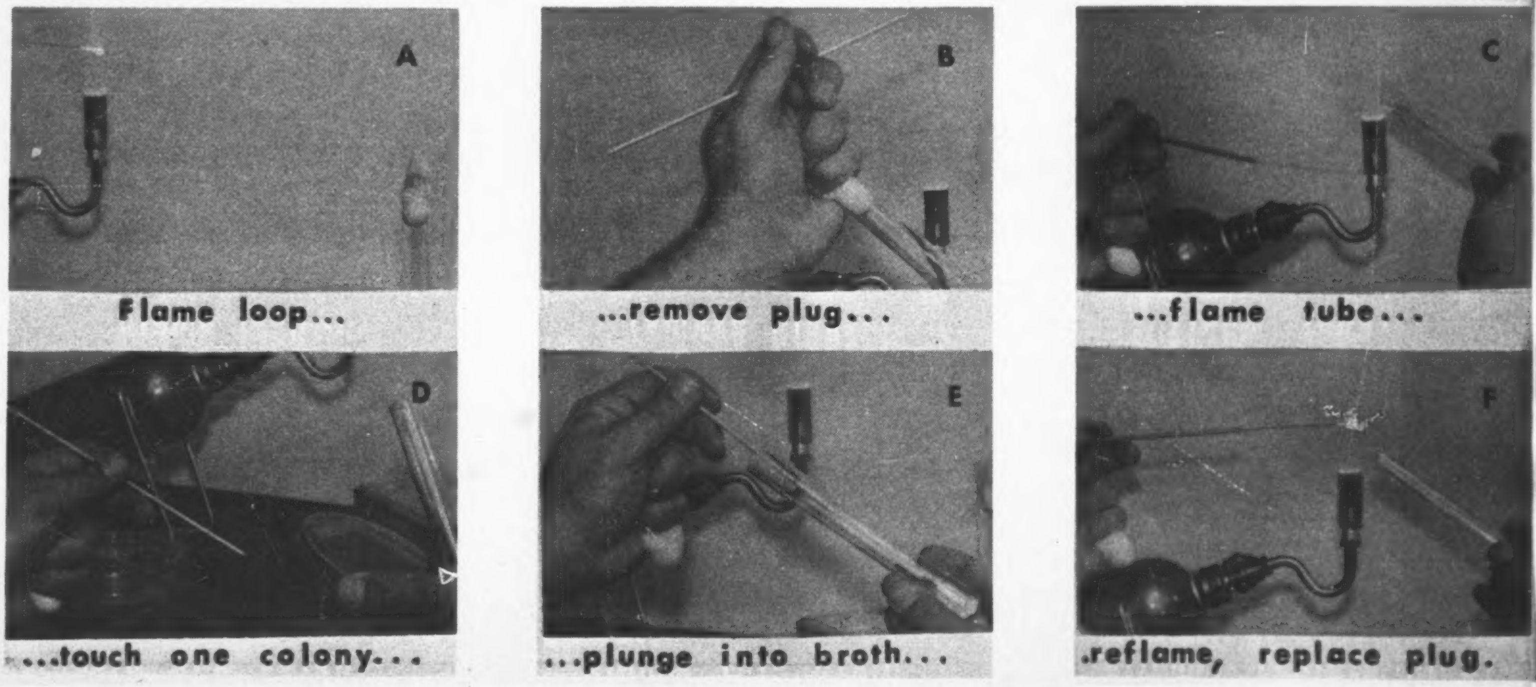
Fig. 3.7
The streak pattern.



4. Flame the loop again before you put it down, to avoid contaminating benches and lockers.

C. MAKE A BROTH CULTURE, of this same strain of bacteria, preferably using bacteria that grow out of the second petri dish. Broth is better than agar for growing large numbers of bacteria after they have been isolated, because broth provides nutrients more readily than agar. To do this, touch a flamed loop to a single colony of bacteria, and plunge it into a tube of sterile nutrient broth. Follow directions in the photographs below. Note: the only way youcan detect motility in bacteria is by observing cells that have grown in broth.

Fig. 3.8
Making a broth culture



Flame loop... ...remove plug... ...flame tube...

...touch one colony... ...plunge into broth... .reflame, replace plug.

D. CHARACTERISTICS OF YOUR CULTURE. You should be able to determine several of the characteristics of your single strain of wild bacteria, such as the following:

1. Description of the colonies as grown on agar.
2. Motility of, or non-motility of the individual cells.
3. Gram positive or Gram negative.
4. Staining with vital dyes and stains.
5. Description and/or drawings of the cells when stained.
6. Rate of oxygen uptake of a broth culture (measured with a small manometer).
7. Sensitivity of antibiotics.

Do as much of this kind of investigating as you have time for, during this week and next week. The above list is only a suggestion. You may find other characteristics you would prefer to investigate.

E. REPORT #2. Write a report on the characteristics of your culture of bacteria. Include where the culture came from, how you isolated the bacteria, and other pertinent information. Follow the proper form in writing the report.

LABORATORY WEEK 4:
ANIMAL-LIKE PROTISTA

This week's work is concerned with the group of living things loosely called "Protozoa": so-called simple, microscopic, one-celled animals. Protozoologists have never been happy with this description, and now welcome a classification system that avoids calling a Euglena or a slime-mold an "animal" or a "plant." According to the newer taxonomy, these forms are all members of the Kingdom Protista, which are living cells that have a nuclear membrane, but are never multicellular (though they may live in large colonies). Protozoa make up a great aggregation of more-or-less animal-like forms, including some that:

1. move and are not green (Phyla Mastigophora, Rhizopoda and Ciliophora),
2. do not move and are not green (Phylum Sporozoa),
3. move and may be either green or not (Phyla Euglenophyta and Chlorophyta),
4. move, are not green, but produce complex spore cases like a fungus (Phylum Myxophyta).

This week we will look at the forms that remind us of animals, and save the plant-like ones for next week.

Though long neglected, the protozoa are now becoming more interesting to biologists, because by virtue of their small size, one can grow large numbers of them in a small space in a completely controlled environment, and thus study life processes on a more accurate scale. A great deal has been discovered in the last few years about the physiology of some of them, and it is surprising to see how much their metabolism is like our own.

General procedure in examining cultures. All nine protozoa named in I and II below are provided in rich living cultures, set up in fingerbowls under binocular microscopes under the window. Using the microscope, you should be able to see the small creatures moving around, make sure that you get some of them into the pipet, and therefore onto your slide. Put about three drops of culture material on a slide, add a coverglass, and examine under reduced light. Please do not cross-contaminate the cultures by moving the fingerbowls, covers or pipets around. You will not be able to tilt your microscope when examining these, because if you do, the liquid culture material will flow off the slide.

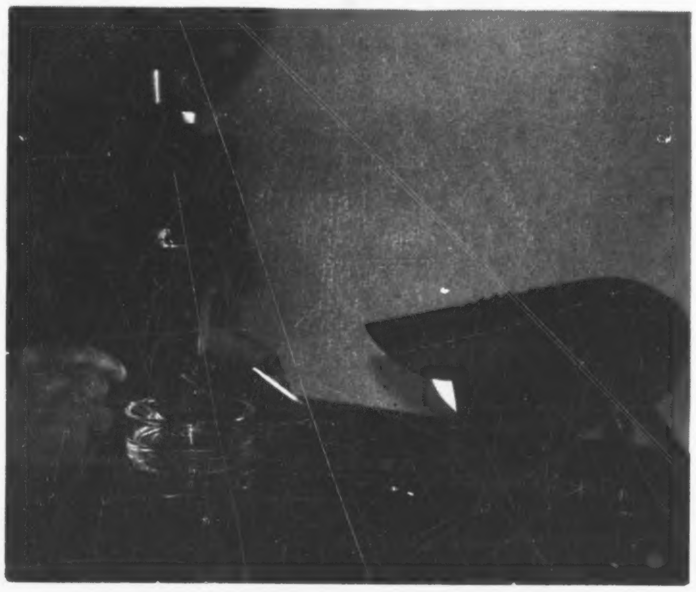


Fig. 4.1 In most cases, you can see the protist cells moving around in the culture when you look through the binocular microscope. Therefore you can be reasonably sure that you get some of them into the pipet and onto your slide.

I. EXAMINATION OF REPRESENTATIVE FREELIVING FRESHWATER PROTOZOA.

A. EUGLENA SP., REPRESENTATIVE OF THE PHYLUM EUGLENOPHYTA. Though both the Euglenophyta and the flagellated Chlorophyta (B, below) move by means of flagella, it is easier to see the Euglenophytes' flagella because they are tinsel type and therefore seem thicker. The Chlorophytes' whiplash type of flagella are more slender than the wavelength of light, and so you can see only the effect they have on the surrounding water, and not the flagella themselves.

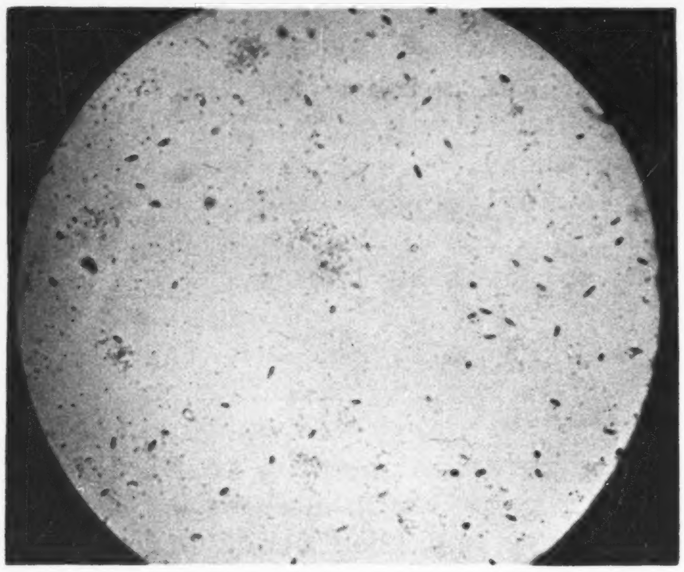


Fig. 4.2 Many Euglena cells, low power.

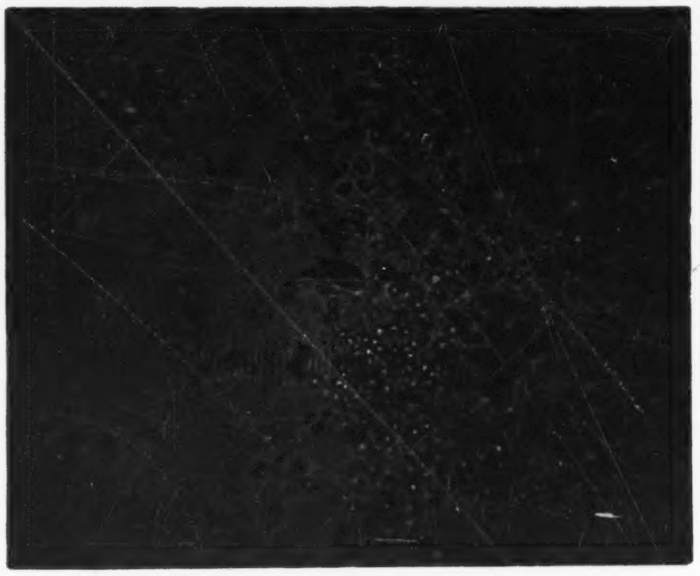


Fig. 4.3 One Euglena cell (arrow), high power.

1. Form. Euglena is an elongate oval, the posterior end may be broadly round or flattened, while the anterior end is more pointed. From the anterior end there extends a conspicuous lashing flagella. A more-or-less solid pellicle covers the cytoplasm, giving a definite shape to the cell.

2. Locomotion. It moves in two ways:

a. When in contact with an object, it may attach itself and crawl or squirm somewhat like a worm. It frequently uses this method to change directions.

b. When swimming freely, the flagella coils around the body and moves the front end of the animal in a circle; giving to the locomotion the appearance of drunken staggering. This is typical flagellate locomotion. However, a few Euglenophytes extend the flagella straight forward and "beckon" themselves forward with the tip of the tinsel flagella.

3. Internal organization. Examine Slide #1 in your slide box as well as the living cells. At the anterior end of Euglena you should be able to find a large reservoir which lies beside the base of the flagella. This will be more apparent if you use vital dyes. You may also see the contractile vacuole opening into the reservoir: a pulsating clear area which is probably active in removing excess water from the cell. Also near the reservoir you will see the bright red stigma or eye-spot, used probably to detect the direction of light rays. Farther back in the

cell, find the nucleus, clear in unstained cells, but it stains well with some vital dyes. The chloroplasts are numerous green bodies scattered in the cytoplasm. What is their function? Look for individuals undergoing longitudinal binary fission, which is the common method of reproduction of many flagellates.

4. Generalizations about Euglenophyta. In II and III below, you will be asked to identify protozoa whose phylum you do not know. You should therefore now begin a list of general characteristics of this phylum, which you can continue as you discover more differences between members of this phylum and others.

B. CHLAMYDOMONAS SP., REPRESENTATIVE OF THE FLAGELLATE CHLOROPHYTA.

Fig. 4.4 Single flagellate Chlamydomonas, high power.

Fig. 4.5 Many non-motile Chlamydomonas, high power.

1. Form. Rounder than Euglena, with two whiplash flagella of equal length.

2. Locomotion. Swims much like Euglena, but does not crawl. You may find many members that have apparently lost their flagella and sit still.

3. Internal organization. You can find the stigma and the nucleus. You may find large cells, not moving, among the others. These are really thick cell walls, inside which the cell is dividing into several young individuals. You will not find separate chloroplasts, but instead you can see a single large pyrenoid which contains the chlorophyll. This structure looks as though it fills the cell entirely, but really it is flat and wrapped around, inside the cell membrane.

4. Generalizations about flagellate Chlorophyta, as in A-4 above.

C. AMEBA PROTEUS, REPRESENTATIVE OF THE PHYLUM RHIZOPODA.

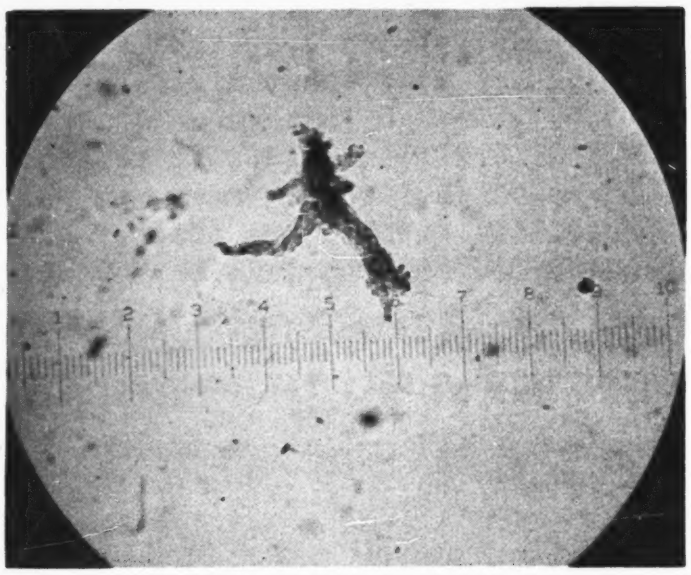


Fig. 4.6 One Ameba proteus cell, as seen under low power. Their large size, definite cell membranes and slow flowing motion should help you avoid confusing them with the ordinary debris in the culture.

1. Form. Ameba has no definite shape, but is irregular and constantly changing. Notice the pseudopodia ("false feet"), which flow out of the cell and are used in crawling and in capturing prey. Find the clear solid ectoplasm on the outside of the cell and the granular endoplasm inside. While you are watching an ameba, move the coverglass slightly back and forth with a needle, to see if the cell is as fluid as it looks.

2. Locomotion. Ameba flows along by projecting its pseudopodia out, and then flowing into them. You will understand this process better if you will make a series of rapid sketches of an ameba that is moving. This process is not very well-understood: some observers think that the body contracts and squeezes out a pseudopodium, while others think that the pseudopodium flows out and pulls the body along behind. What do you think? What evidence do you have for this opinion? As you watch the cells, try to find one that is putting a pseudopodium around a particle of food, and observe the formation of a food vacuole.

3. Internal organization. Examine Slide #2 as well as the living cells. Aside from ectoplasm, endoplasm and food vacuoles mentioned above, about all the definite structures you can see are the nucleus and the contractile vacuole. The nucleus is centrally-located. What shape is it? The contractile vacuole is at the posterior end of a moving animal. Watch the growth of this structure, and see if you can confirm the statement that it is formed by the coalescence of many smaller vacuoles. Estimate the size of the contractile vacuole, and time its rate of contraction, for comparison with that of Paramecium (D, below). Can you find any animals in binary fission? Explain why the fission of Ameba is neither transverse nor longitudinal.

4. Generalizations about Rhizopoda, as in A-4 above.

D. PARAMECIUM CAUDATUM, REPRESENTATIVE OF THE PHYLUM CILIOPHORA. These cells swim freely and rapidly, and will probably be found mainly in a scum at the top of the culture. If you wish to slow them down, make a circle of methyl cellulos on the slide, then put 2-3 drops of *Paramecium* culture inside the circle before dropping the coverglass in place. Those cells that get in the methyl cellulose will be slowed down, but will behave abnormally.

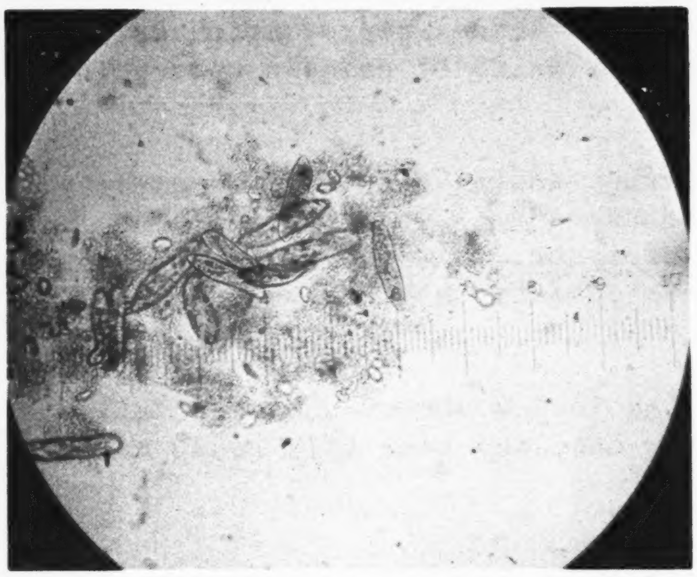


Fig. 4.7 Several *Paramecium*, low power.

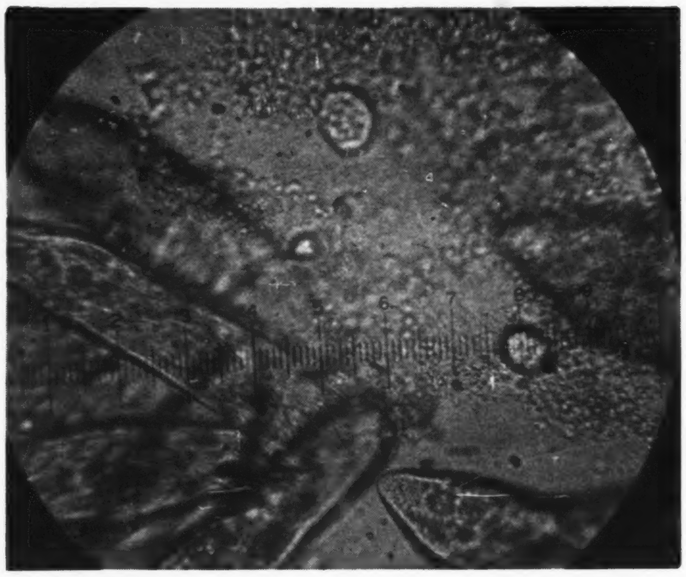


Fig. 4.8 Several *Paramecium*, high power.

1. Form. A rather firm *pellicle* surrounds the cell, giving it a definite shape (Leeuwenhoek called this "slipper-shaped," but maybe he had funny feet). A depression extends from the anterior end of the cell to about the middle, and the posterior portion of this depression is called the *oral groove*. It turns toward the center of the cell and becomes the *gullet*, and then it ends blindly in a *food vacuole*. Under high power, watch particles of India ink as they are pushed down the oral groove, the gullet, and are packed into the food vacuole.

2. Locomotion. About five thousand cilia move the animal swiftly around. They begin in the cytoplasm, extend through the pellicle, and work like oars against the water. The cell rotates as it swims: can you tell which way?

3. Internal organization. Examine Slide #3 as well as the living cells. The *nuclei* are located close to the gullet. Probably what you first see is the *macronucleus*, which is very large, but sometimes you can make out the *micronucleus* embedded in it, especially in stained preparations. (The micronucleus corresponds to the ordinary nucleus of other cells; the macronucleus is a special non-reproductive nucleus found only in the larger Ciliophora.) You should distinguish between the clear *ectoplasm* and the granular *endoplasm*, as you did in *Ameba*. There are two *contractile vacuoles*, one at each end of the cell. You will not find coalescing smaller vacuoles in this cell--instead you will find a complex arrangement of feeding canals apparently leading from all parts of the cell into the vacuole. Estimate the maximum size of the contractile vacuole, and time its rate of contraction. Does it appear that *Paramecium* pumps out more or less water than the *Ameba*? What does this tell you about the permeability of the pellicle of each to water?

Try to find cells in division: they will look like two short plump animals attached together end-to-end, because in the Ciliophora the binary fission is transverse rather than longitudinal. Slide #4 should contain many animals in division stages. If two animals are attached side-by-side, they are not undergoing fission as you might think, but are undergoing a complex kind of sexual reproduction called conjugation. Slide #5 should contain many pairs of animals undergoing conjugation.

After the use of some vital dyes, or after adding very dilute acetic acid and then vital dyes, you may find the animal shooting out hundreds of long tiny threads called trichocysts. Some observers think these are food-catching devices, others do not. What do you think? Any evidence?

4. Generalizations about Ciliophora, as in A-4 above. Complete all four lists at this time, as well as you can, and have them ready to use in II and III below.

II. EXAMINATION AND CLASSIFICATION OF UNCLASSIFIED FORMS.

Using the generalizations made above, determine to which phylum each of the following belongs. Then check, either with the assistant or with reference books, to make sure you have put them in the right phylum. It would be easier for you to do this backwards--find out first, then look at the cultures--but do not do so! This is practice for part III, in which you will have to identify truly unknown forms.

Identify all the following, to Phylum:

A. ARCELLA SP.

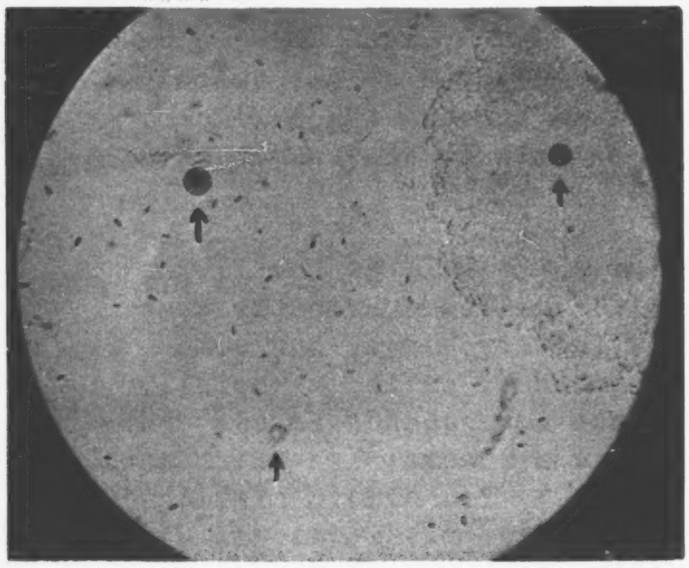


Fig. 4.9 Three Arcella cells, low power. The part most easily seen is the brownish shell, called a test.

top view
side view

Fig. 4.10 Five Arcella cells in a clustered group, high power.

B. BLEPHARISMA SP.

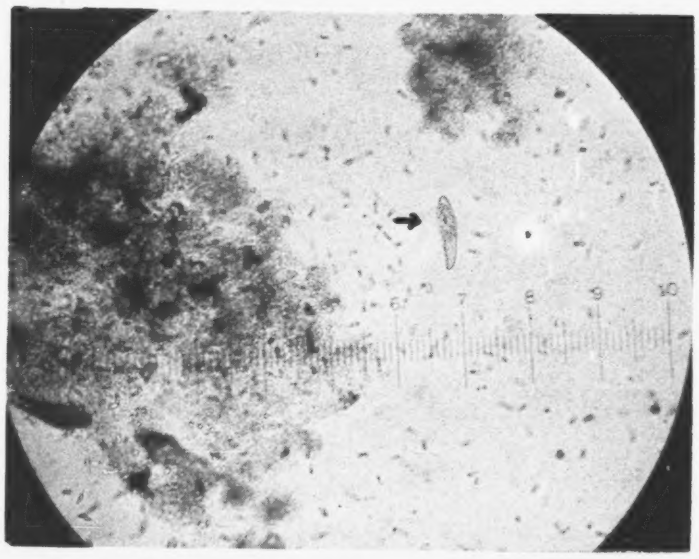


Fig. 4.11
Single Blepharisma cell, low power.

C. GONIUM SP.

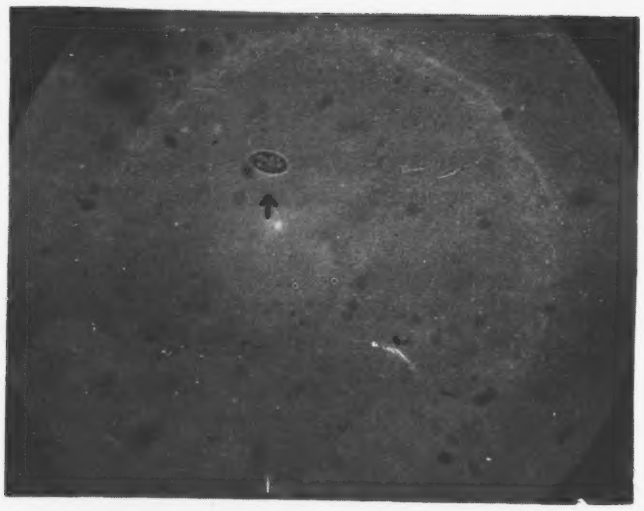


Fig. 4.12 Single Gonium, high power.

Fig. 4.13 Many Gonium cells, high power.

D. PERANEMA SP.

Fig. 4.14
Single Peranema cell, high power.

E. STENTOR SP.

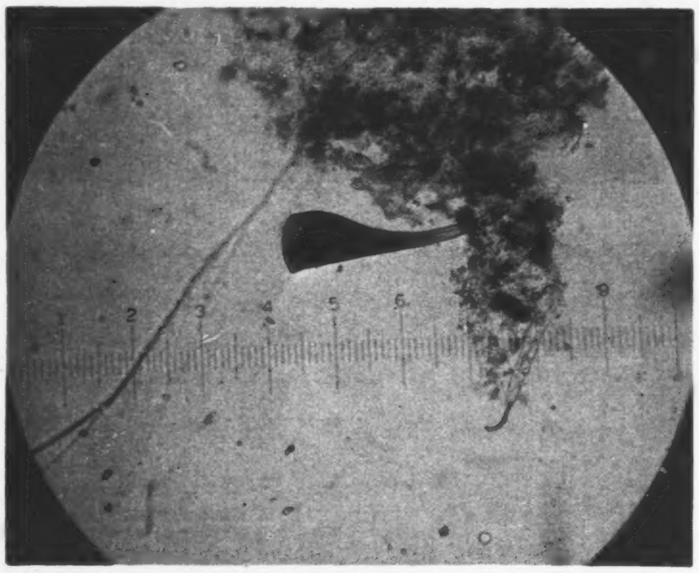


Fig. 4.15
Single Stentor cell,
low power.

III. EXAMINATION OF YOUR WATER SAMPLE.

A. COLLECTING. Take a one-liter bottle and collect a sample of water from some natural area, leaving as short a time as possible between collecting and observing it. Choose lake, pond stream, ditch, or other place where the water has been for some time. Collect not only water, but also bottom sediment and dead or living plants in the water.

B. OBSERVATION. Consider this bottle to be a community, and identify all the living things in it as frequently as you can during the next two weeks. You should be able to identify the bluegreen algae and (perhaps) bacteria from last week's work; protozoa from this week's, and green algae and fungi from next week's. If your sample seems to have little or no life, and does not develop any within two or three days, you should obtain another one. Several books in the library will be helpful to you in identification, and you will find many copies in the laboratory of the pamphlet by Needham & Needham, which will be of greatest help.

This observation should be of the community through time. It would be most interesting if you can work out some method of recording relative numbers of particular species. You may find words like "numerous" and "few" to be adequate, or you may want to work out some more definite pattern of counting. You will find that species that have many representatives will tend to disappear and new forms will appear from eggs or cysts, that were not in your culture to begin with. You may be able to graph your results, after several days, to show increase and decrease in numbers.

Report #3, due in two or three weeks. Write a report of your findings in the community. Make sure that the reader knows the source of the water, how and when you observed it, and exactly what you observed. Obviously you cannot identify everything in the sample, but drawings and/or descriptions of those not identified should be included. After the practice you have had in the first two reports, you should be able to write a clear, concise and accurate report, as a scientist would.

IV. MYXOPHYTA: GROWING AND OBSERVING A SLIME MOLD.

A. SETTING UP A CULTURE. Slime molds normally live in rotting vegetation, and probably devour the bacteria in it, but it is possible to grow them in the laboratory. We will grow a culture of Physarum polycephalum and feed it rolled oats, following the photographed procedure at the end of this section. Take a petri plate of non-nutrient agar, and put a small piece of a growing slime mold in the middle of it. You will find growing slime molds on the front table of the laboratory. With a flamed scalpel, cut out a very small piece of the yellow-colored mold. Keep all your procedure as sterile as possible because, although the slime molds will have bacteria growing in them, we want to keep out other fungi that will overgrow and destroy the ones we want.

In a few hours the mold plasmodium should be flowing across your agar. With flamed forceps put a rolled oat in front of it. Continue to feed it one or two oats at a time until it is very large. If other molds appear, cut them out at once, avoiding spreading their spores. If overgrowth by other fungi seems imminent, make a new culture, using a piece of your culture that is not contaminated.

B. OBSERVATION. Examine the mold with binocular dissecting microscope and with low power of the compound microscope (put the petri dish directly on the stage, but be very careful not to run the lens into the agar). Describe its structure, locomotion, and anything else significant about it.

C. PRODUCING A SCLEROTIUM. Several ways have been described recently for producing this dried, resistant stage of Physarum. It appears that a combination of an abundance of food and fairly dry conditions is best. The culture should become completely dry in not less than 24 nor more than 48 hours. Experiment with some or all of your culture, to see if you can produce a sclerotium.

D. PRODUCING SPORANGIA. These are black spherical bodies, about 1 mm. in diameter, on stalks, that contain many spores. Best conditions for these seem to be light and drying. It is possible that the production of a long day--16 hours or more in length--may help to produce them. Experiment with some or all of your culture, to see if you can produce sporangia.

E. GERMINATING SPORES. If you get sporangia, take one sporangium and mount it on a slide with water. Since this slide will need to last for some time, you may want to put a ring of vaseline on the slide first, then put the sporangium in the middle, add water, and then the coverglass. The vaseline will make the slide fairly permanent against evaporation of the water for a day or two. Crush the sporangium lightly with the coverglass, and then with high power look for the tiny thick-walled spores. After minutes or hours, some of the spores may germinate out of the sporecase, and you might see the tiny myxamebae flowing around. If you see this you are either lucky or very skillful. Check with one of the instructors to find out how to proceed with them. The process of fusion of myxamebae into a new plasmodium has not been observed by very many people.

Report #4, due before the end of the term but not due until you have completed your experimentation and observation of the slime mold. The behavior of your slime mold is unpredictable. Turn in this report whenever your work with it is finished, including what you did and what you observed.

Growing Physarum polycephalum.
An inhabited (yellow-colored) oat grain from a growing culture is transferred to sterile 2% non-nutrient agar.

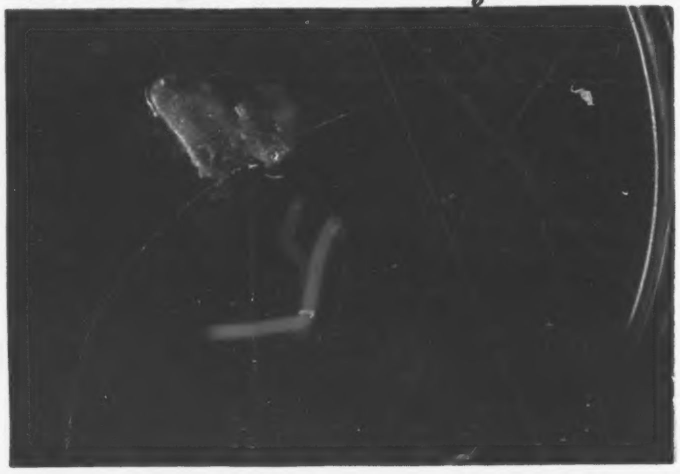


Fig. 4.16 Within 12 hours it begins to move out onto the agar surface.

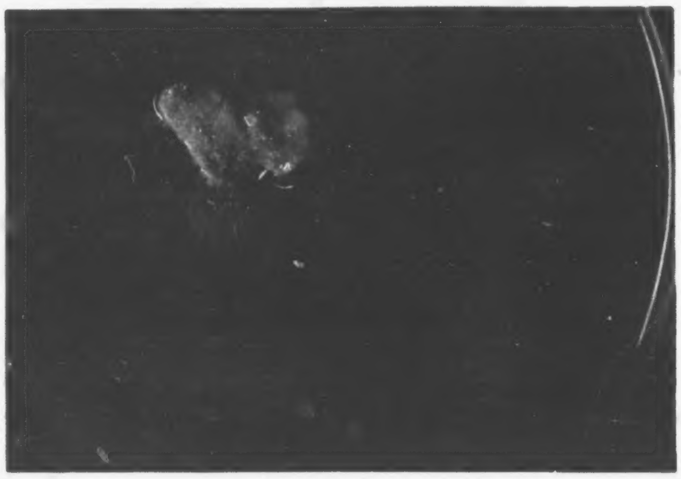


Fig. 4.17 At 18 hours it is almost entirely on the agar.

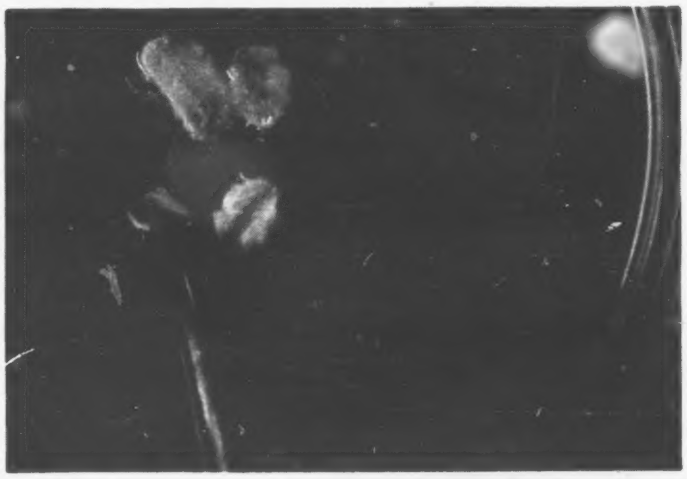


Fig. 4.18 Sterile rolled oat grains are placed in "front" with flamed forceps.

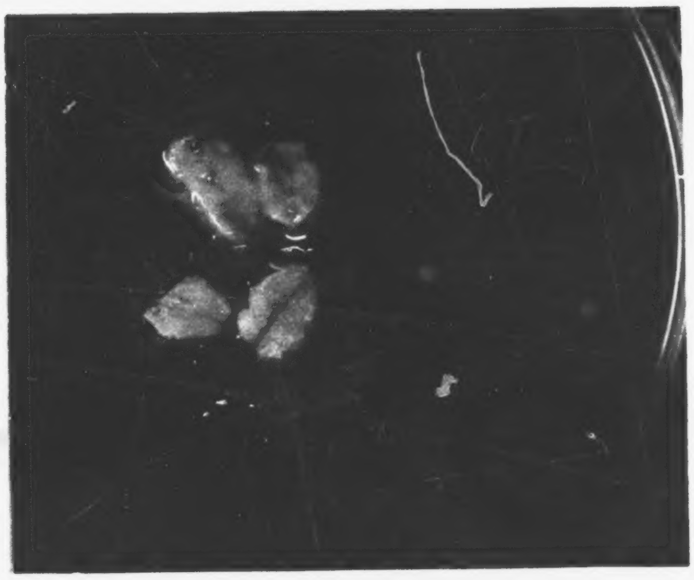


Fig. 4.19 It inhabits those oats, and at 32 hours it has moved beyond them.

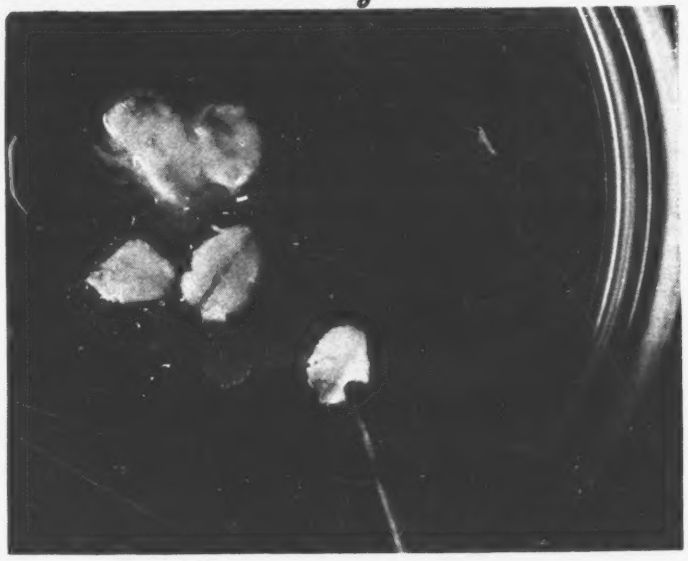


Fig. 4.20 It is fed again, as often as it moves onto the agar.

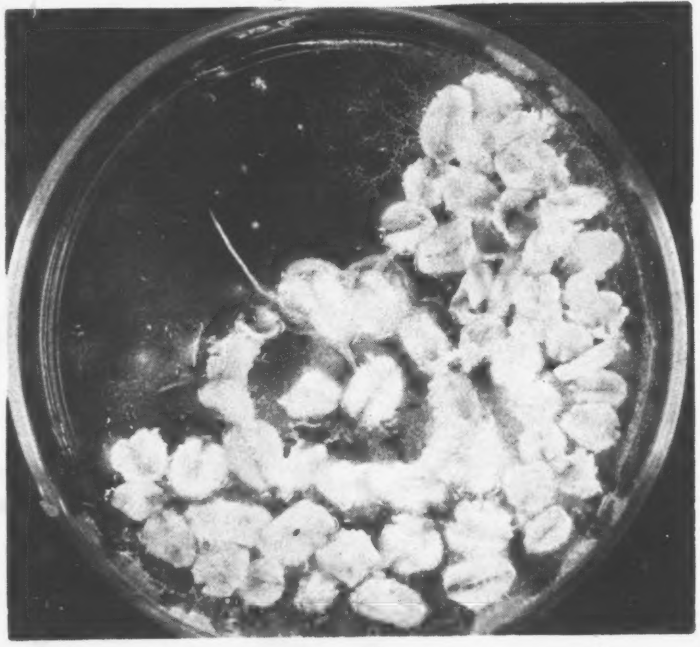


Fig. 4.21 After seven days it nearly covers the agar.

LABORATORY WEEK 5: PLANT-LIKE PROTISTA

Two general groups are taken up this week, loosely called "Algae" (green) and "Fungi" (non-green). Representatives of the following phyla are included: Chlorophyta (other than the flagellate line, studied last week), Chrysophyta, Pyrrophyta, Phaeophyta, Rhodophyta and Mycophyta. Emphasis will be made of the forms that grow in freshwater, in order to help you identify the algae and fungi in your water sample.

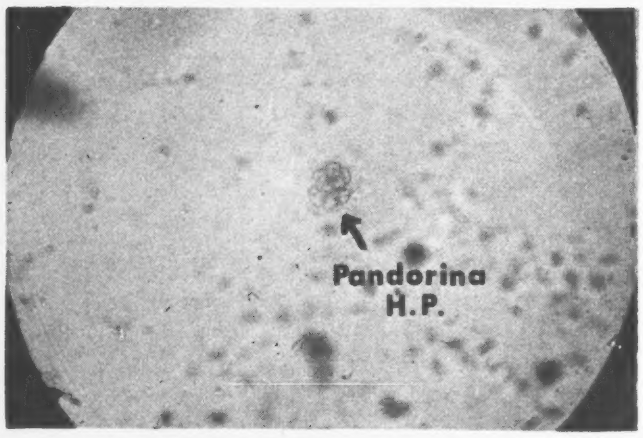
Study the specimens provided in the laboratory, especially trying to see the general characteristics mentioned. Keep good notes and sketches of what you see, so that when you study Chapter 9 in Weisz you can refer to your data book for illustrations of the living things discussed there.

Aquatic cultures are set up under binocular microscopes as they were last week; agar cultures of Mycophyta are on the front table.

I. CHLOROPHYTA, THE GREEN ALGAE.

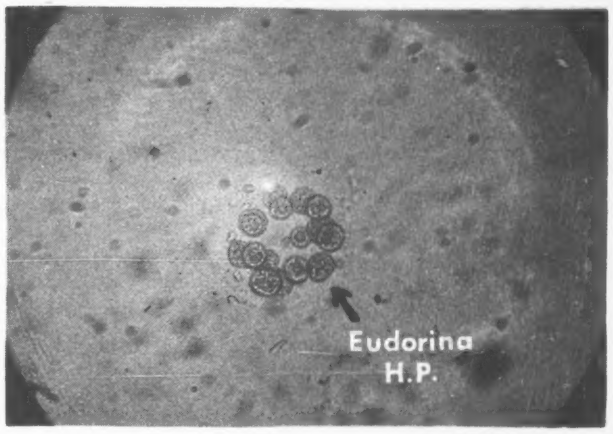
All members of this phylum appear plain green because there is no other pigment in them to mask the natural chlorophyll color; though there are yellow pigments that accentuate the green. Such a negative definition of a phylum will naturally lead to the inclusion of many diverse things, lumped together simply because they all lack any other pigment color. Some members of this group are protozoa. Some members are single-celled or colonial and reproduce by binary fission, but in the larger colonies there are special sex cells that carry on sexual reproduction.

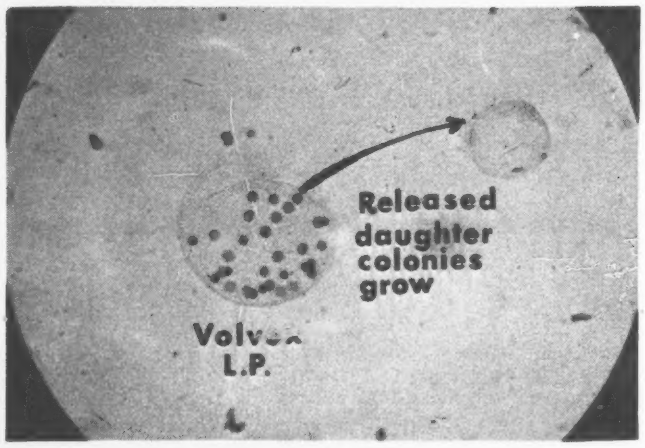
A. COLONIAL FLAGELLATES: PANDORINA, EUDORINA, AND VOLVOX.



← Fig. 5.1

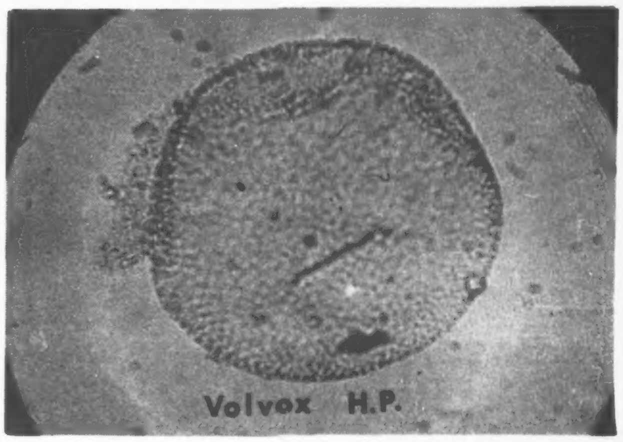
Fig. 5.2 →





← Fig. 5.3

Fig. 5.4 →



First review your notes on Chlamydomonas and Gonium from last week. Then examine each of these cultures under the microscope. Look at the cells and their parts: flagella, stigmata, nuclei, chlorophyll arrangement, etc. When you have completed the studies, you should have an interesting series that illustrates the potentialities of the colonial flagellate Chlorophytes to evolve into multicellular plants. Pay particular attention to the reproductive potentialities of the cells: in the smaller colonies, all cells can divide by fission; but in the larger colonies, only a few cells can do so. Specialization in reproduction is one of the earliest kinds of cellular differentiation. Make sure you understand, however, why these colonies are not members of the Kingdom Metaphyta, but are Protista.

B. A UNICELLULAR COCCOID FORM, CHLORELLA.

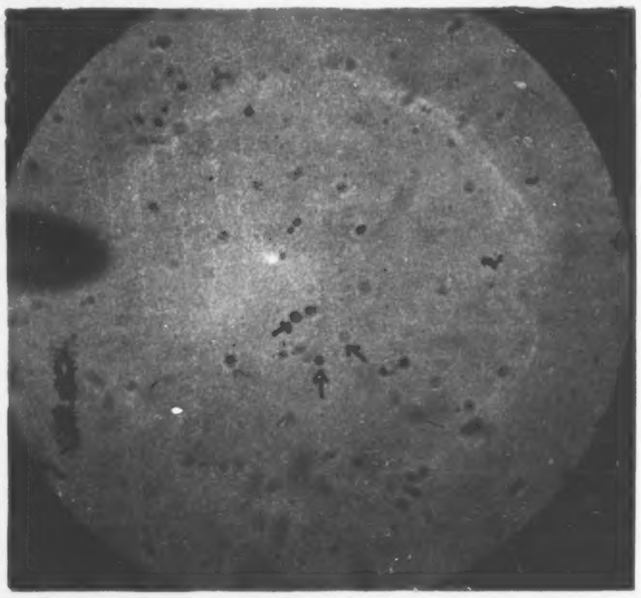


Fig. 5.5
Chlorella, high power.

Examine the culture material under high power, and find as many parts of the cells as you can. Look carefully for cells in the process of division. The term coccoid depends on this process: daughter cells are different sizes.

C. A UNICELLULAR TETRASPORINE FORM, PROTOCOCCUS.

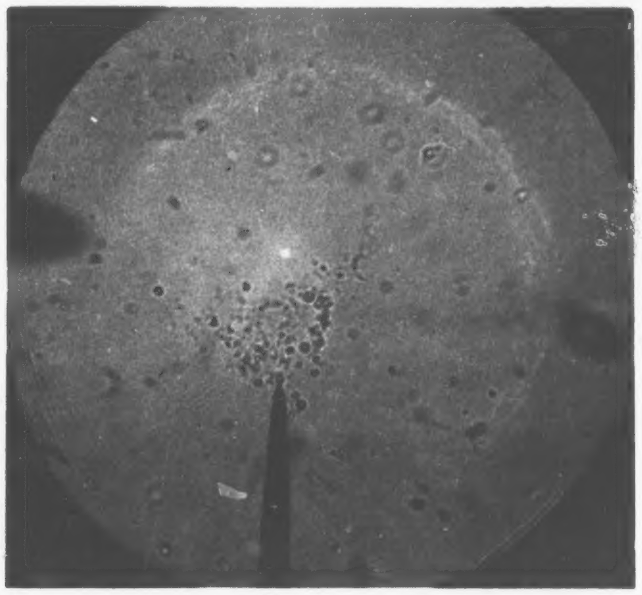


Fig. 5.6
Protococcus, high power.

This alga grows in moist areas such as tree bark, damp soil, etc., but will grow in water. You may be supplied with an aquatic culture of it, or a dry one. If dry, scrape some off the surface and put it on a slide in water with a coverglass.

Compare the cells you see under high power with those of Euglena, Chlamydomonas and Chlorella. Unless you could see the cells dividing, it would be difficult to tell this from Chlorella. Record any differences you find.

(Your own water sample may contain desmids, which are also tetrasporine Chlorophytes closely related to protococcus. Desmids are non-motile, bright green, and usually appear to be made of two cells each.)

D. A FILAMENTOUS TETRASPORINE FORM WITH FLAGELLATE YOUNG STAGES, OEDOGONIUM.

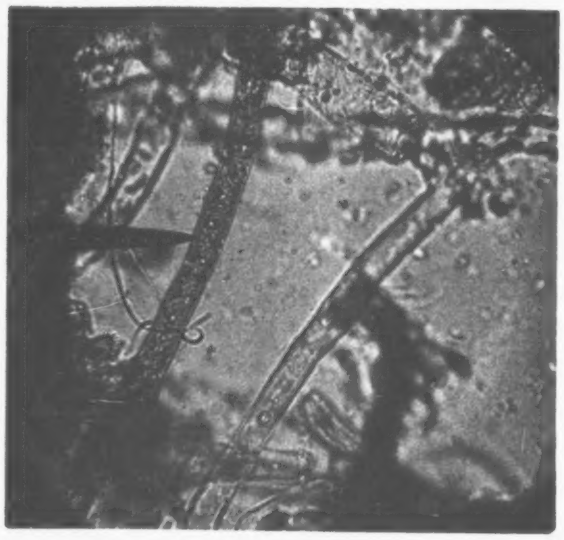


Fig. 5.7
A mass of Oedogonium, with one filament in focus. High power.

Notice that the cells of a filament are not all alike. Find the holdfast at one end of a filament. Some of the cells look empty: flagellate cells have broken out as "zoospores" which form new colonies. Find a cell that is wider than the other cells and somewhat oval in shape. This is an oögonium with a large egg inside. Next find a cell that is the same width as the other cells in the filament, but is quite short. This is an antheridium, and contains flagellate sperm cells when mature. You may find some sperm swimming freely. They are green, ovoid, with a row of flagella around one end. If they find a mature egg, they will penetrate and fertilize it.

E. A FILAMENTOUS TETRASPORINE FORM WITH AMEBOID YOUNG STAGES, SPIROGYRA.

Fig. 5.8 Spirogyra, low power.

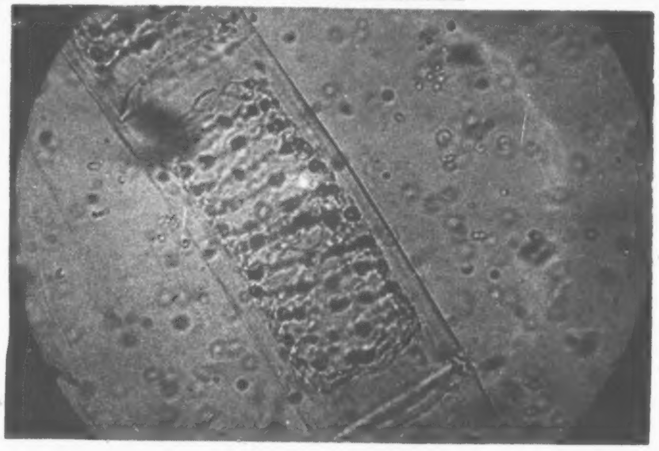


Fig. 5.9 Spirogyra, high power.

Except for those cells undergoing sexual reproduction, all the cells are alike. Examine one cell and find the chloroplast, a ribbon-like spiral green structure with conspicuous enlargements called pyrenoids. Most of the interior of the cell is occupied by a large vacuole (looks empty), and the cytoplasm lines the cell wall and extends as strands toward a central region where the nucleus is located. No zoospores are produced. When two filaments come in contact, two opposite cells may fuse together, and the occupants become ameboid. One of the two will move through the bridge between the cells, and act as a sperm, the other acting as an egg. (Students in our General Biology laboratory have observed this process occurring.) The result of this fertilization, the zygote, develops a thick wall around it and is resistant to adverse conditions.

II. CHRYSOPHYTA, THE GOLD ALGAE.

All algae except the Chlorophyta (above) have, in addition to the chlorophylls, some other pigments that mask to some extent the green color. The Chrysophyta have a brownish-yellow pigment in them, and therefore some people think they look "gold". We will look only at the diatoms as representatives of this phylum.

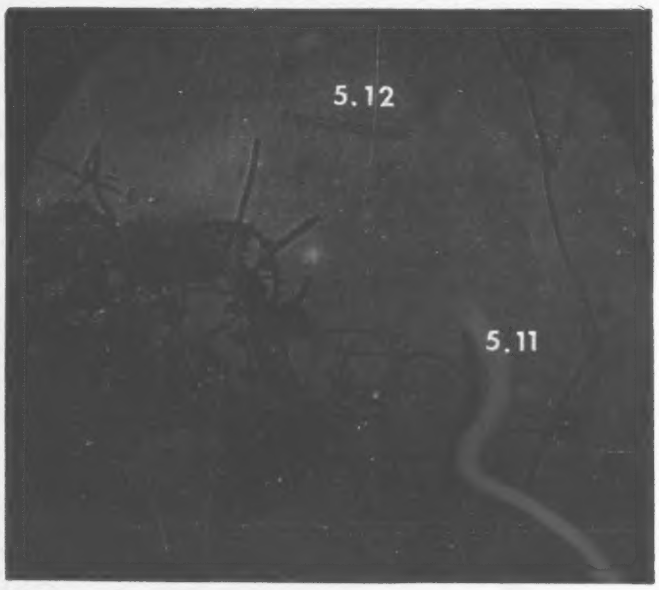


Fig. 5.10
Mixed algae culture, with many diatoms. Low power. See high-power views of two of the cells.

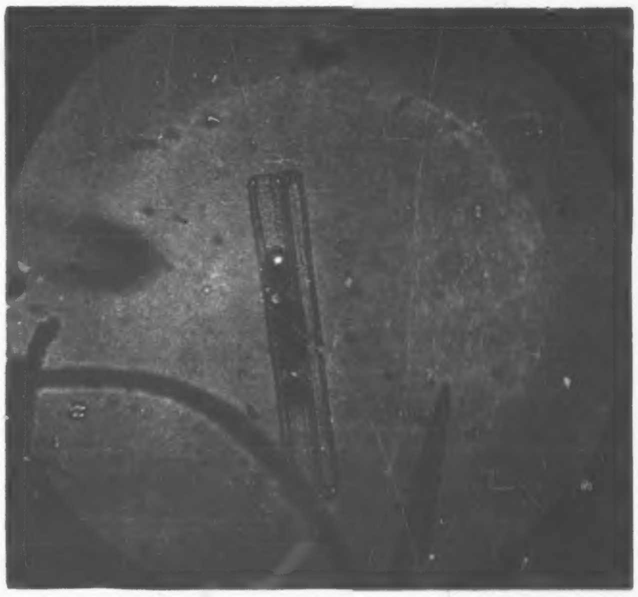


Fig. 5.11
Living diatom cell, high power. Girdle view, showing two valves of the shell.

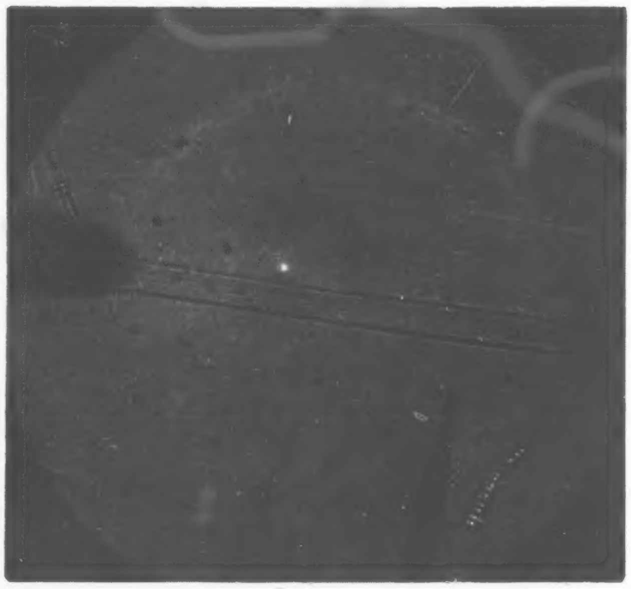


Fig. 5.12 Empty diatom half-shell.

Take material from the bottom of the diatom culture and examine microscopically. This is a mixed diatom culture, so you will find many different forms of them. Notice the hard sculptured cell walls: they have a glassy substance in them made of silica, and they have distinctive, sometimes beautiful, markings. The markings are really openings in the silica shell, through which the cytoplasm can extend and move the cell along. Observe their movement. Find a diatom which can be observed from the top, or valve view. The disc-shaped cells will appear roundish, the long cells will have a groove down the center of the shell. Locate others which can be viewed from the side or girdle view, and see that the shell is made up of two valves, one overlapping the other like two halves of a petri dish. Notice the distinctive "gold" color of the living cells. Also note that many of the shells will have no color at all---these are dead ones, and you see only the shell.

No doubt your own water sample will contain many diatoms, since they make up a large proportion of the plankton food in ponds and lakes.

III. PYRROPHYTA, THE FIRE ALGAE.

The members of this phylum contain, in addition to the chlorophylls, several distinctive yellowish pigments; but their distinguishing characteristic is the possession of two flagella on each cell, at least in the forms you will study. The following are freshwater forms, provided in cultures:

A. CHILOMONAS sp.

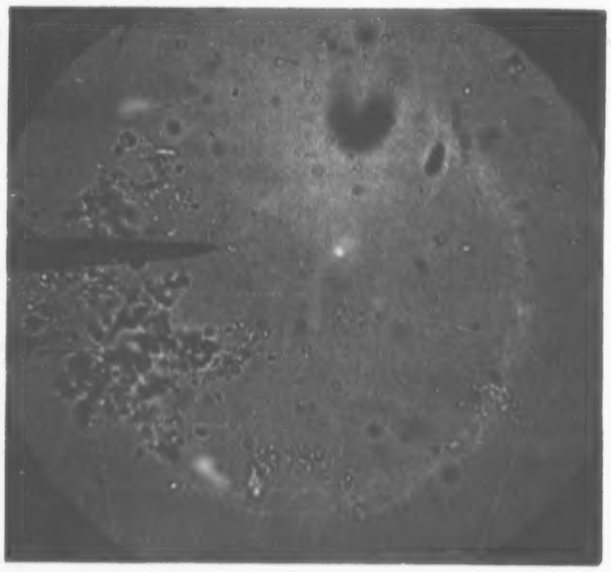


Fig. 5.13
Chilomonas, high power.

Very tiny; study under high power. This bi-flagellate, non-green protozoan is included in this group for reasons that are not clear except for their biochemical makeup. Notice that the flagella do not come directly from the front of the cell, but rather are displaced slightly to one side. Compare this with other flagellate unicellular protista that you have studied. Chilomonas is the food normally included in Ameba cultures.

B. DINOFLAGELLATES.

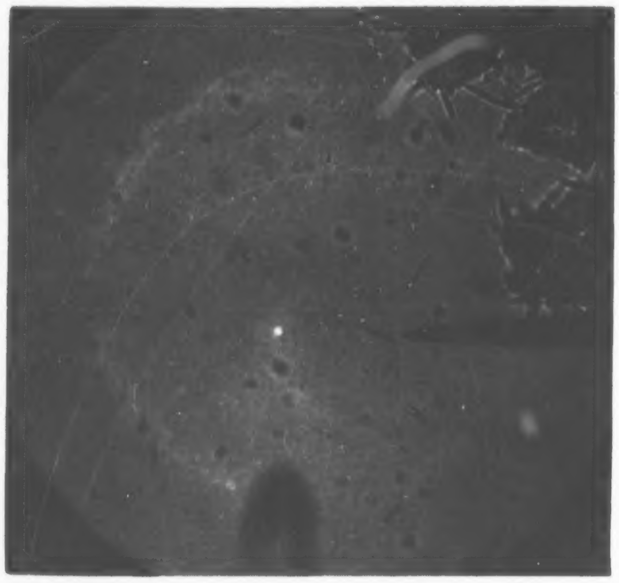


Fig. 5.14
Several different
kinds of
dinoflagellates,
high power.

The culture provided is a mixed dinoflagellate culture. Look for the cellulose cell wall, the two flagella, one in a groove around the middle of the cell, the yellowish-green color, etc. Your own water sample may contain many of these dinoflagellates.

IV. PHAEOPHYTA, THE BROWN ALGAE.

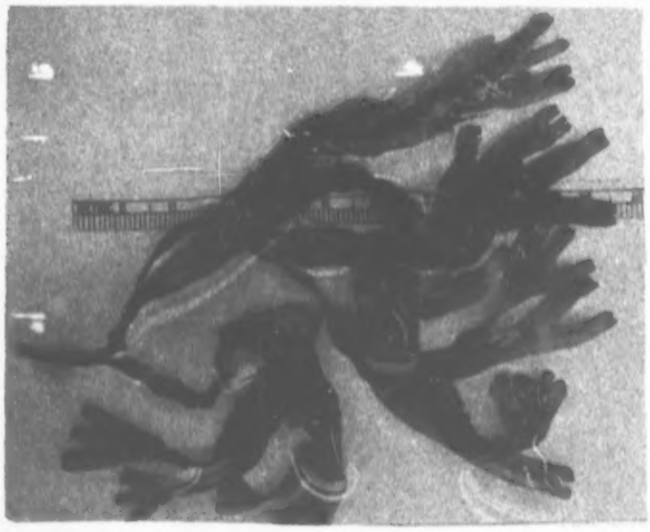


Fig. 5.15 *Fucus.*

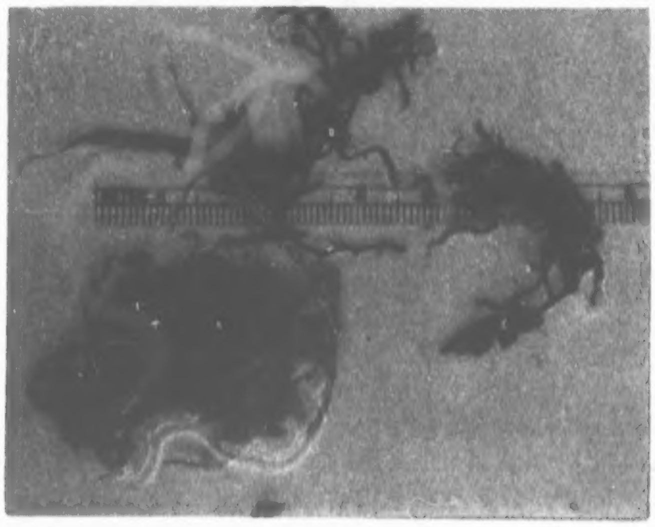


Fig. 5.16 *Sargassum.*

These and the Red Algae (below) are complex marine forms, of little interest to us in our study of freshwater algae. The brown algae have their chlorophyll masked by a brown pigment. Examine the specimens of Fucus and Sargassum in the bottles provided, but do not open the bottles. Look for the following: the body of the plant, made of many cells; sometimes with a holdfast on one end and flat blades on the other; air bladders that serve to float the body. Remember that, although there are various regions to the body, the cells that make them are not specialized except for the reproductive cells. You may find, especially in Fucus, that there are wart-like swellings on the tips of the blades: these are the reproductive organs, and produce the eggs and sperms.

V. RHODOPHYTA, THE RED ALGAE.

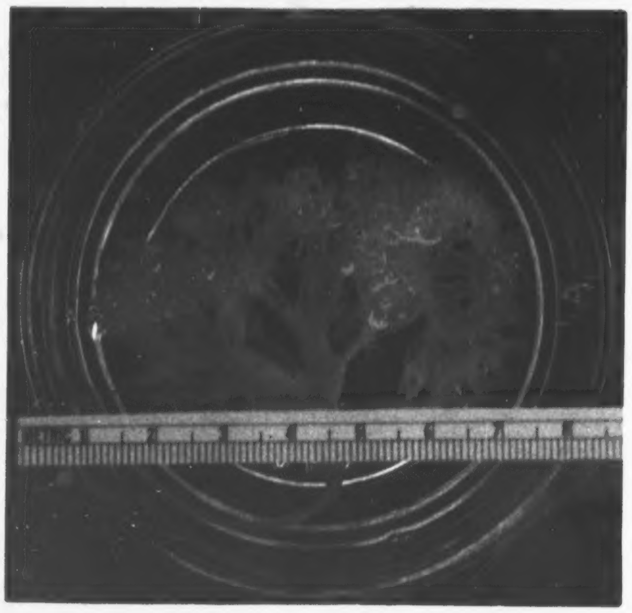


Fig. 5.17
Chondrus crispus.

The pigments that mask the chlorophyll in these protists are mainly red ones, though bleached out by the preservative used. Examine the specimen provided of *Chondrus crispus*, and compare with that of *Fucus*.

VI. MYCOPHYTA, THE FUNGI.

These are the protista that, for the most part, behave more like plants than like animals, but have no chlorophyll and therefore must depend upon outside sources for their food. This is a negative definition, and thus leads to the inclusion of an array of different kinds of living things, of which we can show you only a few common examples.

A. SAPROLEGNIA, AN AQUATIC MEMBER OF THE PHYCOMYCETES.

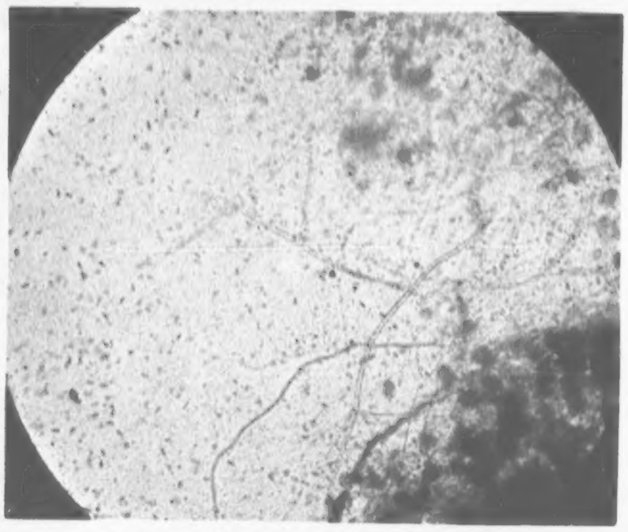


Fig. 5.18 *Saprolegnia*, low power.

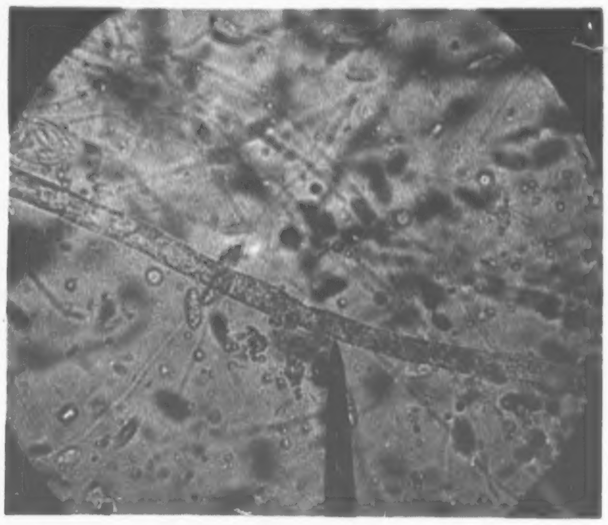


Fig. 5.19 *Saprolegnia*, high power.

Put a small piece of the mold culture on a slide with water. Since this is a very pale species, you will probably want to use some vital dye with it to make it show up better. Notice the lack of chlorophyll, but its general similarity to the filamentous algae. It produces zoospores in much the same way that *Oedogonium* does, and its sexual reproduction resembles that alga also. Notice particularly that the mold is made up of long filaments called *hyphae* that have many nuclei but do not have cross-walls between the nuclei. This is a typical Phycomycete characteristic, not found in the other fungi.

B. PHYCOMYCES BLAKESLEEANUS, A TERRESTRIAL MEMBER OF THE PHYCOMYCETES.

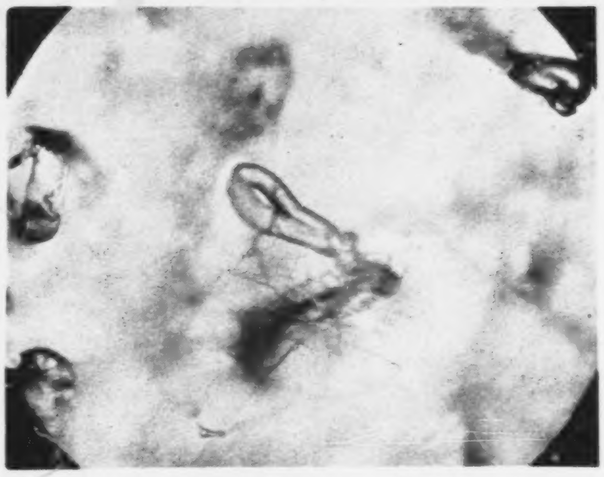


Fig. 5.20 P. blakesleeanus, low power. Some ordinary hyphae plus two joining ready to mate.

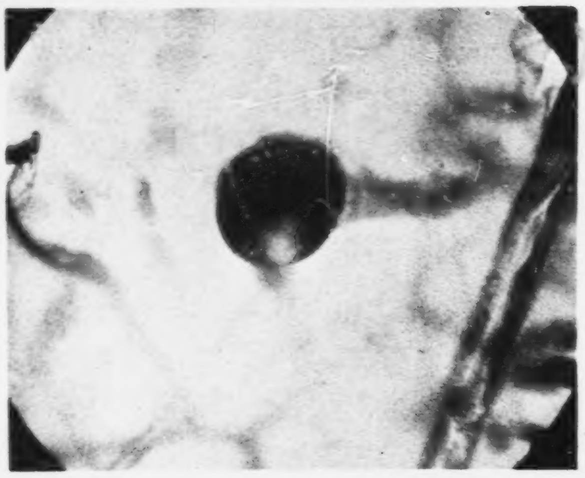


Fig. 5.21 P. blakesleeanus, low power. Zygospore (black) resulting from sexual reproduction.

This fungus is provided on agar. Grow a culture of it yourself on mycological agar and watch the formation of its reproductive bodies. Inoculate one side of a petri dish of nutrient agar with a spot of spores from the "+" strain of the fungus, and the other side with "-" strain. Grow the fungus, cover down, in your desk drawer, and observe its growth. Observe the hyphae germinating from the spores. (You can watch the growth on the petri dish by putting the whole dish on the stage of your microscope and focusing with low power. Do not use high power.) Refer to the whole mass of hyphae as a mycelium. Watch the line where the two strains grow together, for the formation of zygospores. How does this process compare with that of Spirogyra?

C. ASPERGILLUS SP., REPRESENTATIVE OF THE ASCOMYCETES. Both Ascomycetes and Basidiomycetes have hyphae with cross-walls dividing the nuclei from each other, while the Phycomycetes (above) do not. Cultures of Aspergillus and Penicillium are provided already growing on petri dishes of nutrient agar, and you should examine them both with binocular and compound microscopes.

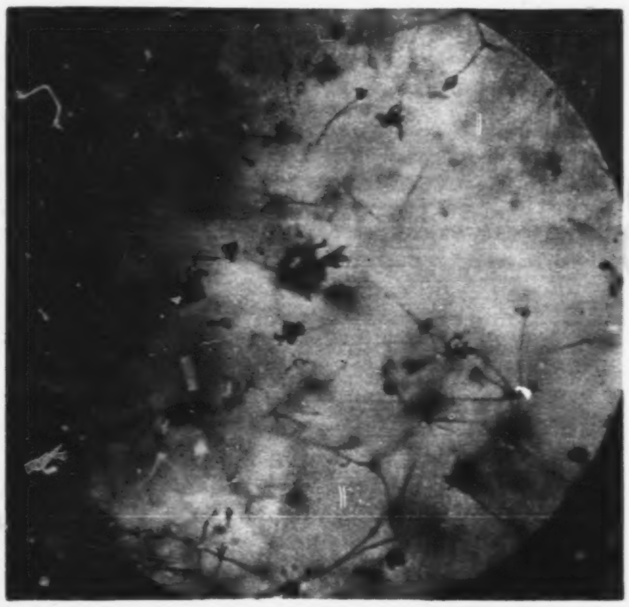


Fig. 5.22 Aspergillus sp., low power as it grows on agar. Sporophores show here, but no perithecia.

Examine the mycelium and find the occasional club-shaped sporophores with the very tiny asexual spores produced inside them. Now look for larger dark-colored bodies (produced later than the sporophores), the perithecia, which contain the sexual spores. Remove one of the perithecia with a flamed scalpel, mount it on a slide with water, and crush it gently with a coverglass. A number of clear cigar-shaped bodies should be squeezed out of the perithecium, visible under high power. These are asci. Vitally stain the asci and count the number of ascospores inside. The production of asci is characteristic of the Ascomycetes only---Basidiomycetes produce a similar but different structure when producing sexual spores.

D. PENICILLIUM SP., A SECOND REPRESENTATIVE OF THE ASCOMYCETES. The two genera Aspergillus and Penicillium are responsible for most of the rotting of foodstuffs and clothing that we encounter, but are valuable in commercial production of cheese, flavorings, acids, etc.

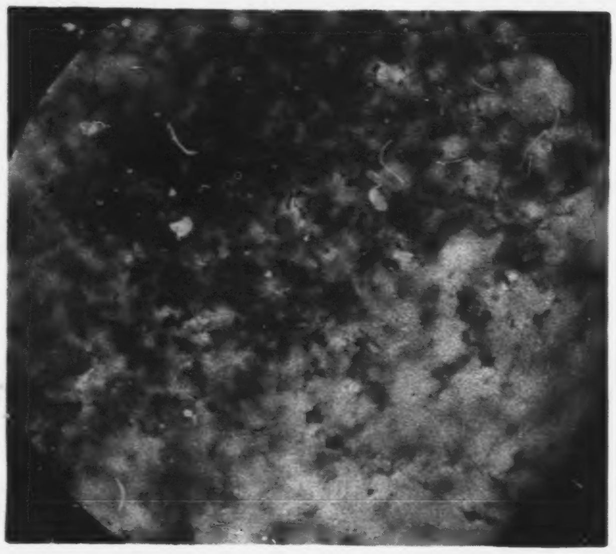


Fig. 5.23
Penicillium roqueforti,
low power as it grows
on agar.

Look for sporophores in the mycelium---they look like tiny brooms, and the bristles of the brooms are rows of asexual spores. Compare the hyphae with those of Aspergillus. You will probably not find any perithecia in this culture, because although Penicillium is an Ascomycete, it seldom undergoes sexual reproduction and therefore seldom produces asci and ascospores.

E. SCHIZOSACCHAROMYCES OCTOSPORUS, A UNICELLULAR ASCOMYCETE. This form is a yeast: tiny, single-celled protista that require a wet environment. Most yeasts do not produce asci even though they are Ascomycetes, but this species has been selected because it may do so under the right conditions.

Fig. 5.24
Schizosaccharomyces
octosporus,
high power. Notice
that some of the
cells almost look
like hyphae.

Scrape a small bit of the growth of yeast off the agar and put it on a slide with a drop of water. Examine under high power of the compound microscope, finding the small cells. With a vital stain, locate the nucleus. You will probably find some of the cells in division. What green alga does this asexual reproductive process remind you of? Look carefully among the cells for larger structures, the asci, the result of sexual reproduction. How many ascospores are inside? Is this a general rule, or does the number vary?

F. COPRINUS SP., REPRESENTATIVE OF THE BASIDIOMYCETES. Coprinus is a mushroom: certainly not an ordinary Basidiomycete, but one at least in which you can see the basidiospores. Though most Basidiomycetes are parasites of plants, this one lives saprobically in the soil. Occasionally it send out a spore-bearing structure, a mushroom, that produces and disseminates the spores. Bear in mind that most of the mushroom's mycelium is underground.

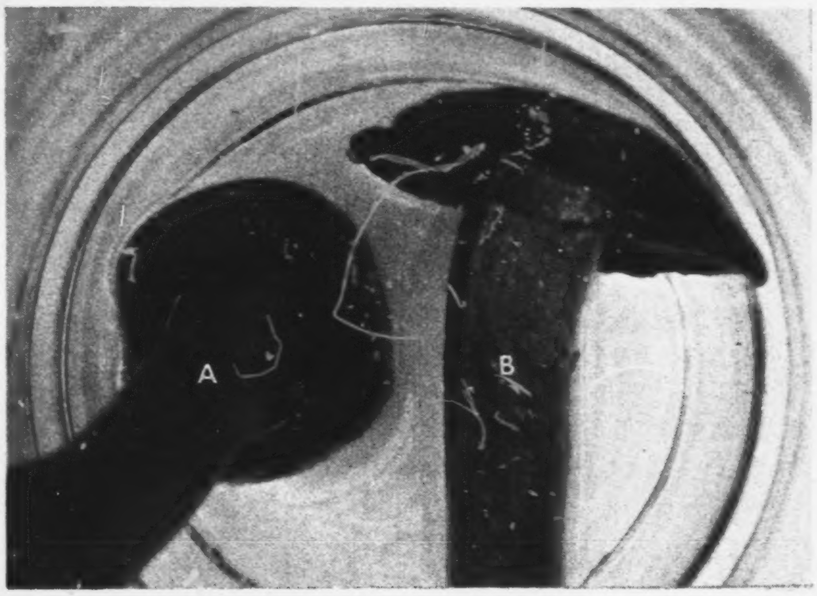


Fig. 5.25
Two specimens of Coprinus; A shown from below to show gills, B sectioned longitudinally.

Examine a specimen of Coprinus, and find the following: the stem-like stipe, the cap-like pileus, and the gills on the lower side of the pileus.

Examine the demonstration slide, a section cut through the gills of Coprinus. Find the edge of a gill under high power, and you will see the rows of basidia that produce the basidiospores. An ideal section will show you four basidiospores on each basidium. In what way do basidia resemble asci? How do they differ from asci?

(Note: although many Basidiomycetes produce asexual spores as well as basidiospores, mushrooms do not do so.)

VII. LICHENS, MIXTURES OF ALGAE AND FUNGI.

Lichens are really double protists: both a fungus and an alga living in such close and permanent relationship as to be considered a single "plant".

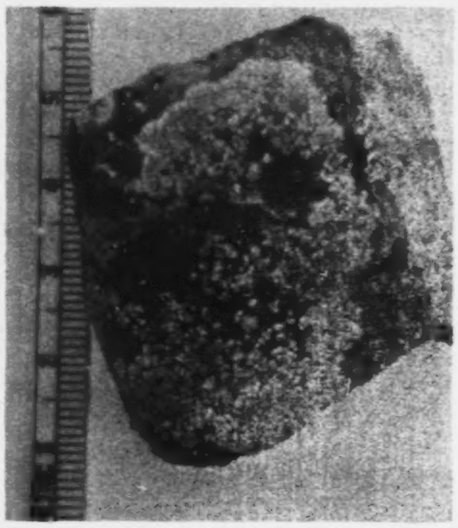


Fig. 5.26
Crustose Lichen.

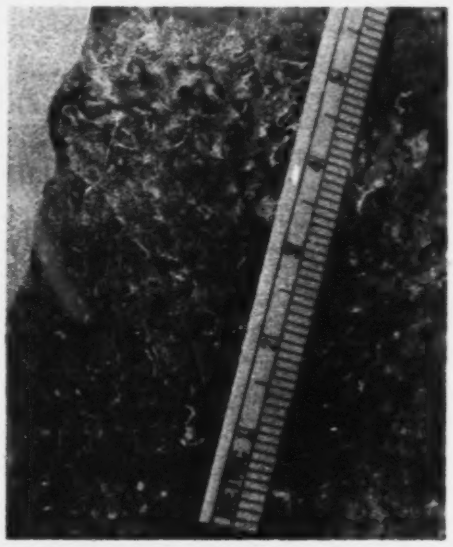


Fig. 5.27
Foliose Lichen.

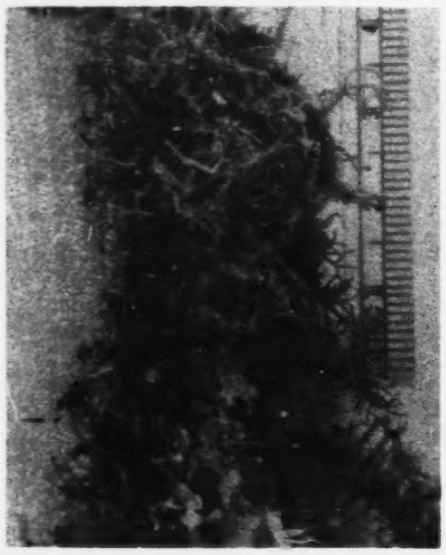


Fig. 5.28
Fruticose Lichen.

Examine the bottled and dried specimens on demonstration and note that they come in a variety of shapes, depending on how close they are to the substrate upon which they grow. Crustose means close; foliose means farther away and leafy-appearing; fruticose means even farther away and branching.

Look at the demonstration slide of a lichen, stained to show which is alga (inner part) and which is fungus (outer part). What advantage can you see to each, in this arrangement, and in this relationship?

LABORATORY WEEK 6:
PORIFERA, CNIDARIA AND CTENOPHORA

The work for the following two weeks is kept intentionally short, so you can have the flexibility you will need to carry out the research for Reports #3 and #4. Materials for Laboratory Weeks 6 and 7 will be in the laboratory for two weeks, and you can organize your time to complete it around your research. In particular, you will probably want to get an early start on the experimentation for planaria regeneration, Report #5.

The two weeks' work is designed to let you look at a large array of unfamiliar metazoan animals, so that in studying Weisz's Chapter 11 you will be familiar with some examples of the forms discussed, and you can understand the text more fully.

These creatures have very little in common with each other, so each group will be introduced separately. Their main attribute in common is the undeveloped condition of the middle layer of the body, the mesoderm, and the lack of a cavity in this middle layer, which if present would be called a coelom. As you progress through the two weeks' work, watch especially for these two characteristics. The sponges have no mesoderm at all; the Cnidaria and Ctenophora have no real mesoderm but have a mesoglea that takes its place; the flatworms have a true mesoderm but have no coelom; and the roundworms have a cavity that looks like--but is not--a true coelom. Though these characteristics are not obvious on the outside of the animals, they have a great significance in the evolutionary history of the metazoa.

I. PORIFERA, THE SPONGES.

The sponges are very different from better-known animals--so different that some biologists would like to give them a special sub-Kingdom of their own within the Metazoa; but for our purposes we need merely to assign them Phylum standing. The bodies of sponges are essentially double-walled sacs, perforated with many tiny holes through which water is pulled, with a large hole through which water goes out. Sponges are food-strainers. Through a process of inward and outward pocketing and folding, most sponges have made their bodies so complex that the basic pattern is obscured. The animals you will see here will demonstrate the progressive specialization of sponges into better and better strainers.

A. A SIMPLE SAC SPONGE, LEUCOSOLENIA SP. Obtain a Syracuse watch glass from the laboratory assistant that has two kinds of preserved sponges in it--this one and the next one, Scypha sp. Scypha looks like a small prickly vase; Leucosolenia doesn't look like anything organized.

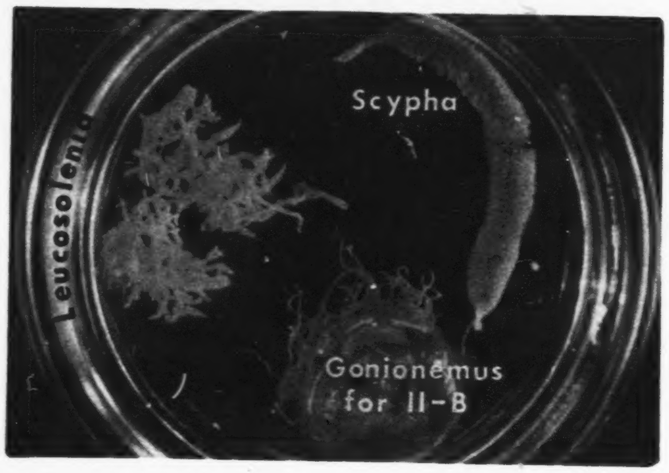


Fig. 6.1 Three animals in watch glass.

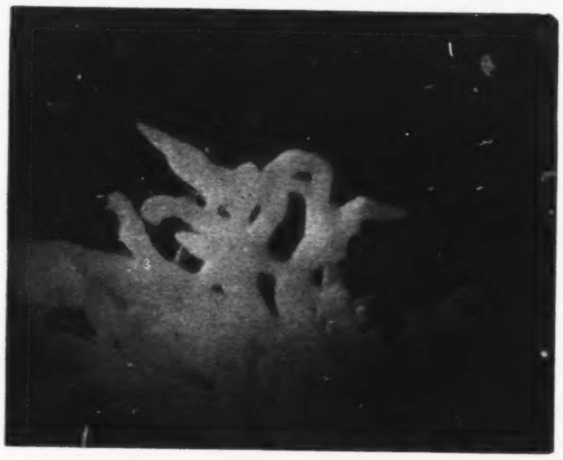


Fig. 6.2 Leucosolenia, x20.

In life Leucosolenia is a colony of fine, slender, attached sacs, somewhat finger-shaped, with no true symmetry whatever. Examine the colony carefully under high power of the binocular microscope. The entire body is covered with tiny incurrent pores, through which water and food are pulled by a current of water. The outer end of each member of the colony contains a fairly large excurrent pore, through which water, minus the food, leaves the body.

B. AN INTERMEDIATE SAC SPONGE, SCYPHA SP. (= Sycon, = Grantia). Under the binocular microscope, the body of this sponge looks full of holes, too, and these holes are also called incurrent pores; but as you will see in the prepared slide, these are not homologous to the same-named holes in Leucosolenia. Find the top opening in the animal, the excurrent pore. Notice that the body is firm and rigid. It is held in shape by an interlaced network of spicules, some of which you can see sticking out through the body wall.

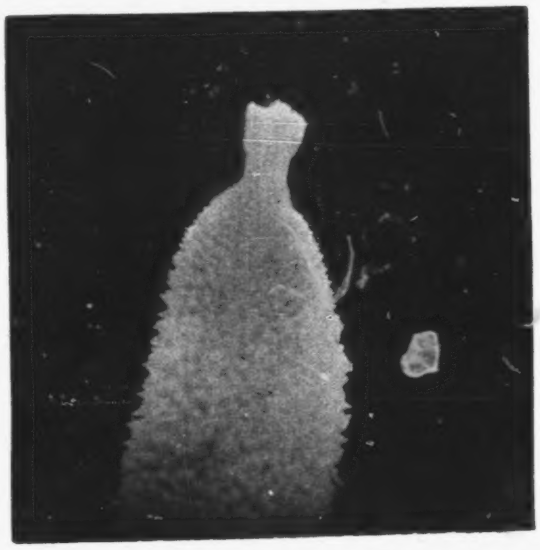


Fig. 6.3 Scypha, excurrent end on "top", x20.

Fig. 6.4 Scypha, longitudinal section through excurrent end, x10.

Examine slide #6, a cross-section through Scypha, first with the binocular and then the compound microscope. Compare it with the demonstration slide of a longitudinal section through Scypha. In the demonstration, find the excurrent pore. In the cross-section, notice that, although the body wall appears thick, it is actually extremely thin; the thin membrane is thrown into complicated folds due to in-pocketings of the outside and out-pocketings of the inside. What you called (above) the incurrent pores were really the openings of the in-pockets. The true pores (which correspond to the incurrent pores of Leucosolenia) are openings in the true thin body wall. You can probably see a few of these if you look carefully. You will not see the spicules in these slides because they were dissolved out during slide preparation. If you could, you would see that most of them lie in a narrow space between the two layers of the thin body wall.

Examine the inner lining of the out-pocketings carefully, with high power, and be on the lookout for flagellated cells that have high stand-up collars around the bases of the flagella. If you are lucky enough to find any, you will have seen the typical diagnostic cell of the sponges, which both makes the current of water, and devours the food. The many other kinds of cells present in the sponge are mainly structural, and live off the bounty of the collared flagellate cells.

C. COMMERCIAL SPONGES

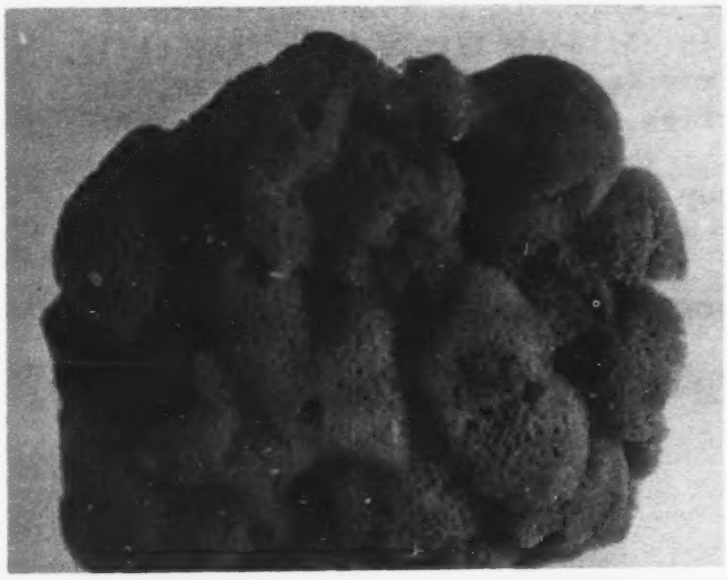


Fig. 6.5 Whole dried commercial sponge.

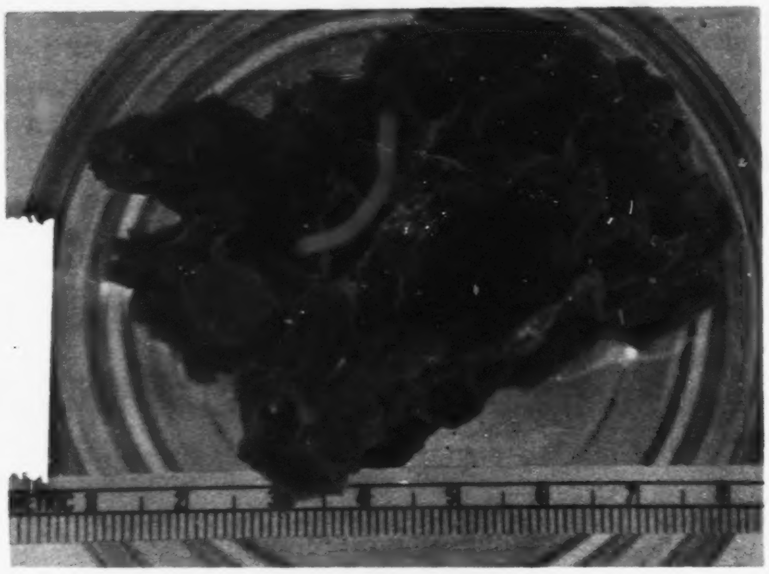


Fig. 6.6 A piece of preserved sponge.

Examine both the preserved and the dried specimens on the laboratory tables. In the dried ones, only the skeletons are left, like the spicules described above. The soft tissues have rotted away. Feel the skeleton as you examine it. In the preserved specimens, the soft tissues are still present. Note that you are not seeing a whole sponge, but just a piece of one. Try to make out the complicated network of channels that water must go through in order to be strained. Can you imagine the complex folding and pocketing that had to take place, to evolve from Scypha to this?

D. COMPLEX NON-COMMERCIAL SPONGES. Several skeletons are on demonstration, in the wall case in the hall; to show you the varied shapes that are assumed by the supporting networks of most marine sponges.

II. CNIDARIA; THE ANIMALS WITH NEMATOCYSTS.

Some of these animals are attached (sessile) forms, and others float; but they all have several characteristics in common:

1. They are radially symmetrical.

2. They have only one opening into the gut, which acts both as mouth and anus.

3. None has a true mesoderm.

4. All have special stinging and/or entangling cells called nematocysts.

5. All have an outer layer and an inner layer of the body wall, the ectoderm and the endoderm.

Keep these general characteristics in mind as you look at each specimen in this phylum, and make sure you know how each member displays the characteristics.

The ectoderm and endoderm are usually only a single cell-layer thick. More highly organized animals (from Platyhelminthes onward) have a layer of mesoderm between these layers, derived from the endoderm and giving rise to the greatest bulk of the body; but in the Cnidaria and the Ctenophora (III below) there is only mesoglea in this position, which normally contains little bulk, few cells, and is derived from the ectoderm. Mesoglea may be very thin as in hydras, or enormously thick and gelatinous, as in jellyfish.

All members of this phylum can have their body plans reduced to one or the other of the following body forms:

1. Attached form, called the polyp form or hydroid generation;
2. Floating form, called the jellyfish form or medusoid generation.

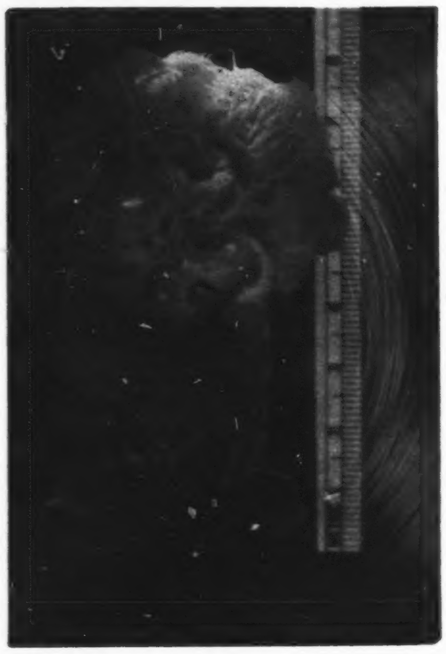


Fig. 6.7 Sea anemone, a polyp form.

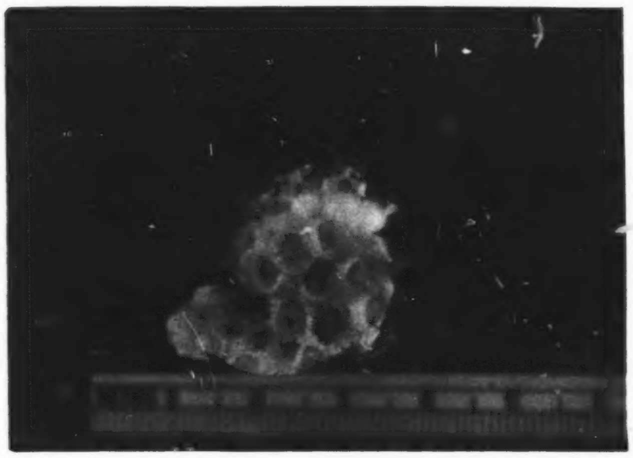


Fig. 6.8 Colony of corals, polyp forms.

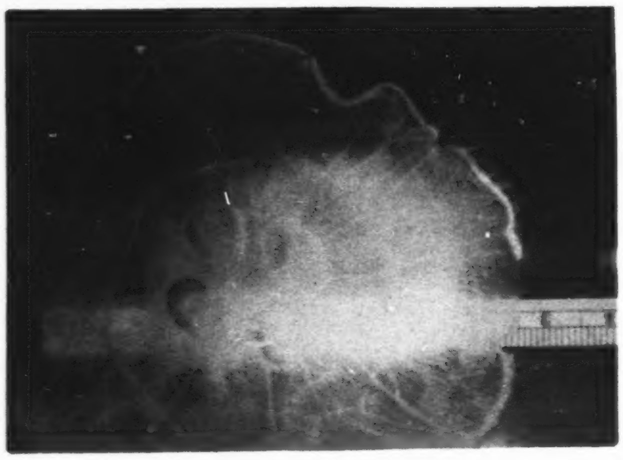


Fig. 6.9 Aurelia, jellyfish form.

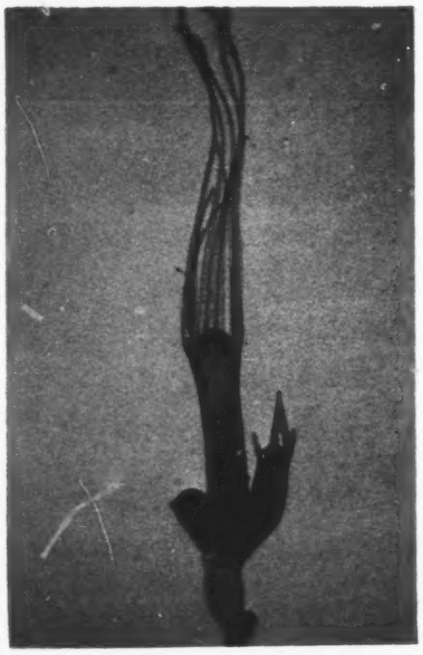
Fig. 6.10 Polyorchis, jellyfish form.

The reason that we mention generations is that many species of Cnidaria alternate these two body forms from generation to generation: a hydroid produces a medusoid produces a hydroid, and so on. The alternation of generations is called metagenesis.

A fairly common characteristic among Cnidaria is the tendency to form colonies--that is, an individual buds off from another individual, asexually, and instead of separating, the two remain attached in such a way that they share any food through a common gut. In species of marine Cnidaria, huge numbers may be so attached.

A. A HYDRA, PELMATOHYDRA OLIGACTIS.

Fig. 6.11
Pelmatohydra oligactis, preserved specimen with two buds, x20.



1. Living animals. On your table you will find a stack of culture dishes containing living hydras and living planarias. Take one dish, and when finished with it please return it to the stack at once to prevent drying out. Examine the hydra in the dish with the dissecting microscope, both high and low power. You should see the basal disc by which it is attached to the glass, the stalk extending from the basal disc to the body, the tentacles (how many?) radiating from the top of the body, and the mouth in the middle of the bases of the tentacles. The raised area around the mouth is the hypostome. The anatomy will be seen better in 2 below. Your specimen may have a bud--a young animal produced asexually--attached to it. Where do buds develop? Do buds have all the body parts that adults have? Do all the tentacles appear to develop at the same rate? Observe the reactions of the hydra to various stimuli, and if possible watch it catch and eat some prey. Does the animal seem to react as a single, well-organized being, or as a collection of cells or parts?

2. Preserved whole mount. Examine the anatomy described above, in slide #7. You should be able to see through this whole mount, and discern that there is a cavity in the animal. Does it appear that this cavity extends into all parts of the body? How many openings does this cavity have to the outside? How does this compare with a sponge? With a frog? You should refer to this digestive cavity of the hydra as the enteron, or gut.

Look for "warts" on the tentacles. Using low power, examine one of the warts. They are made of special large cells called cnidoblasts, and each cnidoblast has a bristle extending from it, called the cnidocil. When the cnidocil is stimulated by taste and touch, it acts as a trigger to discharge the nematocyst inside the cnidoblast. By careful focusing you may be able to distinguish unexploded nematocysts inside the cnidoblasts--they look like tiny light bulbs with their bases toward the cnidocil.

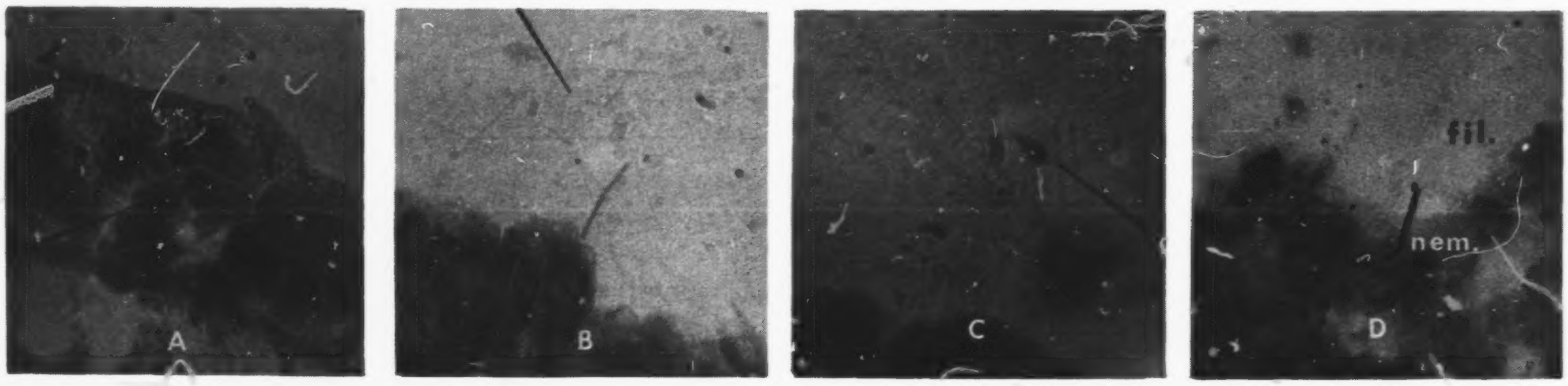


Fig. 6.12 Nematocysts, as seen under oil immersion (x970). A: unexploded nematocysts, in cnidoblasts, within hydra ectoderm, stained. B: exploded nematocysts, filaments of entangling type. C: adhesive type. D: Light-colored stinging type, nem.=nematocyst, fil.=filament.

3. Exploding the nematocysts. Put a living hydra on a slide, add a coverglass, and squash the hydra thoroughly. Add a cytoplasm-staining vital dye, and examine along the tentacles under high power. Move the slide around and see that there are several different kinds of nematocysts. You should distinguish at least two general types:

   a. The large penetrating variety, flask-shaped after discharge, with powerful barbs at the neck of the flask. How long are the filaments that project from the necks? Are they all the same length? These are filled with a paralyzing poison, and may penetrate and paralyze prey.

   b. The smaller varieties, oval in shape, with filaments that have no barbs. Some have long entangling filaments, others have short adhesive ones.

Remember that nematocysts are diagnostic for the Cnidaria: all Cnidaria have nematocysts, any animal that has nematocysts is a member of the Cnidaria.

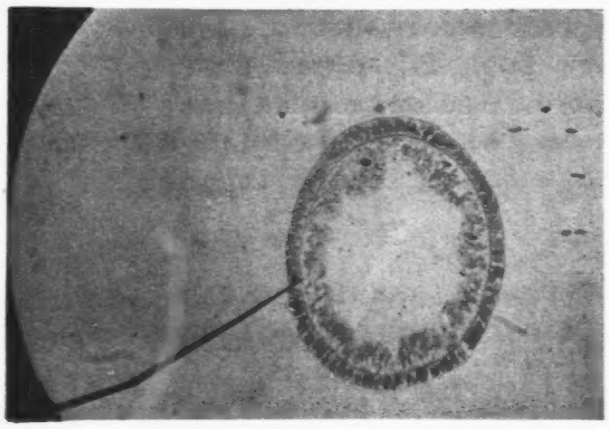
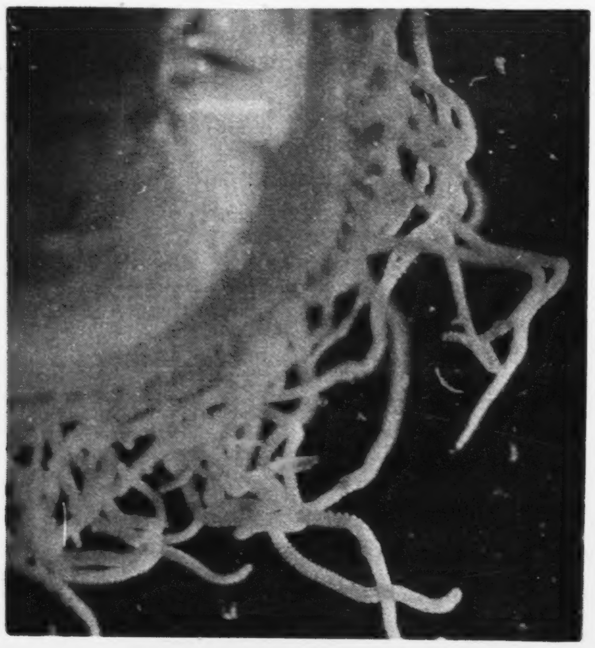


Fig. 6.13 Cross-section of hydra, low power. Pointer is indicating the mesoglea.

4. Cross-sections through the body of hydra. Examine slide #8 under low and high power. Find the ectoderm, endoderm, and the very thin mesoglea between them. What do the individual cells look like that make up these layers? Are they all alike in shape and location? Remind yourself of the active motions made by the living hydra. Where do you suppose the force comes from that moves the animal?

B. A JELLYFISH, GONIONEMUS MURBACHII. This preserved animal is in the watch glass with the sponge specimens. You will need to turn it from one side to the other to see all the parts.

The jelly-like appearance is produced by an enormous development of the mesoglea; the ectoderm and endoderm are not much thicker than they are in the hydra. In any radially-symmetrical animal, you must not refer to dorsal, ventral, etc., but only to oral (mouth) side and aboral (non-mouth) side. The convex "upper" surface of the umbrella is the aboral surface; the concave "lower" surface is the oral surface. The mouth is on the end of a finger-like projection, the manubrium, on the oral side. What is the shape of the mouth opening? A short canal leads the food from the mouth through the manubrium into the four-cornered stomach in the center of the umbrella. From each corner of the stomach extends a radial canal to the edge of the umbrella. All radial canals meet the single ring canal that runs around the edge of the umbrella.



← Fig. 6.14
View from aboral
side of Gonionemus,
x20.

Fig. 6.15 →
View from oral side
of Gonionemus, x20.

The most active parts of the body are located near a branch of the digestive system: the coiled gonads (ovaries or testes) lie on the oral surface of the umbrella, one under each radial canal. The tentacles and sense organs lie near the ring canal. Make sure that you see the warts on the tentacles that contain the cnidoblasts and nematocysts. Also note the knee-pad-shaped adhesive discs on the tentacles that help the animal hold onto things.

C. A COLONY OF POLYPS, OBELIA SP. First look at the demonstration in the hall case, of a preserved colony of Obelia, to see how it grows.

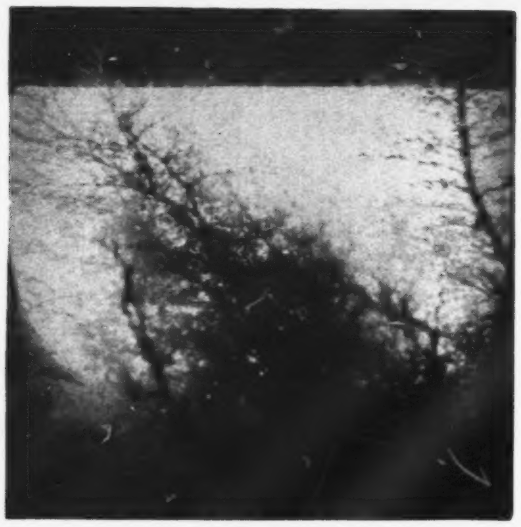


Fig. 6.16 Obelia colony, life-sized.

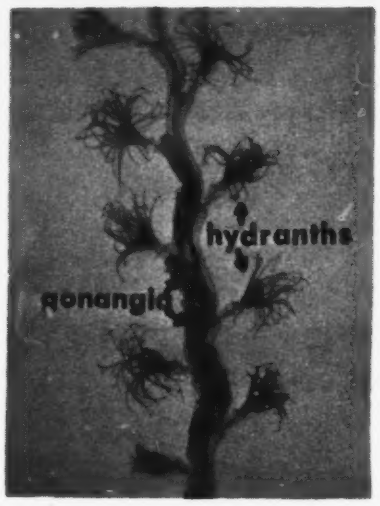


Fig. 6.17 One stalk of Obelia colony, x20.

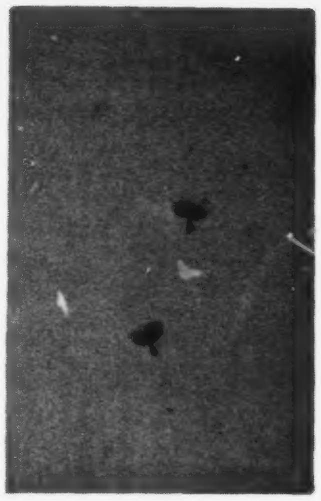


Fig. 6.18 Full-grown Obelia medusae, x20.

The material mounted on slide #9, Obelia whole mount, is only a small piece of one of the large colonies. The colony resembles the structure that would result if the buds of a hydra were to remain fastened to the parent instead of detaching. All individuals remain on the parental "stem." You will, however, find two different kinds of polyps:

a. The hydranth polyps, or feeding polyps, are the most numerous. These are fundamentally similar to a hydra. They have hypostome, mouth, tentacles, enteron, and nematocysts. How many tentacles? Is there a stalk? Is there a basal disc? Try to follow the enteron, to confirm that it extends from one individual to the next without interruption. The whole colony is covered on the outside with a transparent covering called the perisarc, and the hydranths can pull down inside the perisarc when disturbed.

b. The gonangia polyps, or medusa-bearing polyps, are less numerous than the hydranths. Do they occur in any regular position? These individuals consist mainly of a central stalk (= body with no mouth), covered with a swollen perisarc. On the stalk you can see growing tiny medusa buds, which will be either male or female individuals. When mature, the medusae will break loose, go out through the opening at the tip of the perisarc, swim away from the parent colony, mate and die. Fertilized eggs will then produce another colony of polyps. This clearly demonstrates the metagenesis of Cnidaria.

Examine the microscopic demonstration of a mature Obelia medusa, and notice its similarity to Gonionemus.

D. DEMONSTRATIONS OF OTHER CNIDARIA. Look at the labelled demonstrations in the display cabinet in the hall, of other kinds and shapes of Cnidaria. In each one, make sure that you know what you are looking at:

Whether it is a whole animal or merely a shell; whether it is a polyp or a jellyfish; whether it is a colony or a single individual. If you do not understand them, ask for help.

III. CTENOPHORA, THE ANIMALS WITH COMB PLATES.

Fig. 6.19
A "sea gooseberry", life-sized. Anterior end to the right. Needle indicates a comb plate. Only one of the two tentacles is in view.

Until recently, the Ctenophora were combined with the Cnidaria, as classes of the phylum Coelenterata. Taxonomists came to feel that these were better-understood if separated from each other, since the Cnidaria have nematocysts and the Ctenophora do not; and so the phylum Coelenterata was eliminated and the humerous-sounding class names were elevated to phylum standing. Though odd, the names do have meaning: the Greek roots are cnido- = thread; cteno- = comb.

Examine the demonstration in the hall case of a "sea gooseberry." These animals are almost, but not quite, radially symmetrical: they have two retractable tentacles that make them partly bilateral. Notice the rows of comb plates that run in the oral-aboral direction. The comb-plates possess patches of ciliated cells, that move the animal around. All the Ctenophora are marine, of little importance to us, although it is said that they devour large numbers of newly hatched ocean fish.

LABORATORY WEEK 7:
PLATYHELMINTHES AND NEMATODA

I. PLATYHELMINTHES, THE FLATWORMS.

These are all bilaterally-symmetrical worms that contain a true mesoderm rather than the mesoglea found in Cnidaria and Ctenophora. The presence of the mesoderm makes possible the development of true muscles, and therefore these worms can be more active than animals that are less well-organized. Associated with the increased activity is the development of a head end with sense organs concentrated on it. Fossil history shows that at one time in the earth's history there were large numbers of non-parasitic animals like our present flatworms, but now there are few because of the competition by more efficient animals.

In order to understand the body plan of the flatworms, you will first study the common free-living planaria, a member of the Class Turbellaria.

Then, to see the results of greater and greater specialization toward parasitism, you will examine a fluke (Class Trematoda) and then a tapeworm (Class Cestoda). In looking at the parasitic forms, notice especially the adaptations that fit them for their mode of life: holdfast organs, suckers, hooks, etc. You will find that the digestive system is reduced in size and importance; the musculature is reduced; the sense organs are reduced; but there is a great increase in the size and complexity of the reproductive systems. Many flatworms, especially the flukes, have a very complex life history involving several intermediate hosts, and a definitive host in which the parasite becomes sexually mature.

A. A PLANARIA, DUGESIA SP.

1. Living animals. Living planaria will not be available until the beginning of the seventh week. Take a Syracuse watch glass, add a small amount of well water or pond water (not tap water) and then pick up a planaria with the large-mouthed dropping pipet provided. Do not allow the worm to stay in the pipet even for a brief moment--if you do, it will stick to the inside and you will not be able to get it out at all. Examine the worm with a binocular microscope.

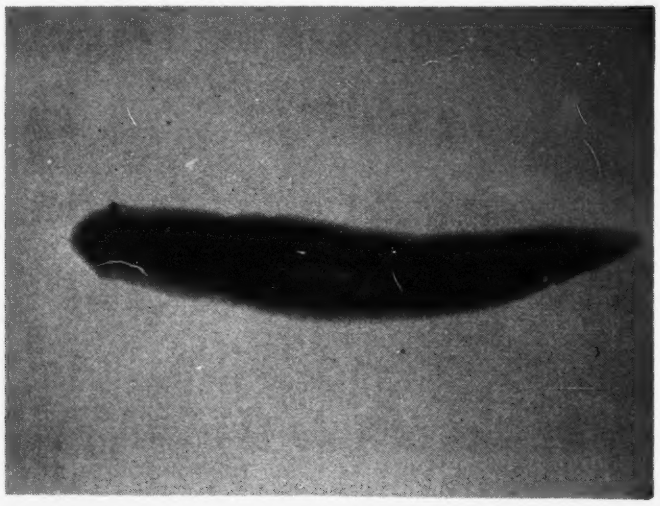


Fig. 7.1
Dugesia sp. x10.
Anterior end to the left.

Notice the head, and the bilateral symmetry. The head contains two light-sensitive eyes, and two auricles which are not ears but rheotactic organs that detect the direction of water current. The mouth is not on the head, but is located about halfway back on the ventral surface, on the end of a muscular tube-like pharynx that is normally housed in a sheath but can be pushed out when feeding. You may be able to watch the feeding process, by offering the animal a very tiny piece of liver (freezing compartment of refrigerator). Do not leave un-eaten liver in the watch glass with the animal more than a few minutes.

The planaria moves in two ways. The normal gliding is brought about by the action of ciliated cells on the ventral side of the body. The crawling motion is accomplished by the contraction of layers of longitudinal and circular muscles inside the body. Can you tell at which end the waves of contraction begin? Can you stimulate the animal to make it reverse this normal response?

2. Whole microscopic mount, slide #10. Examine first with the binocular microscope, then with low power of the compound microscope. Before the animal was killed, it had been fed dyed egg yolk so you could see where the digestive system is located. The most obvious part of the system is the pharynx, which is clear of food but muscular. Find the three branches of the gut, all of which are attached to the anterior end of the pharynx. There is one anterior main branch and two posterior main branches. There are also many secondary branches from the main branches. Food is distributed to all parts of the body, even before it is digested. Digestion takes place within the cells that line the gut, as in the Cnidaria.

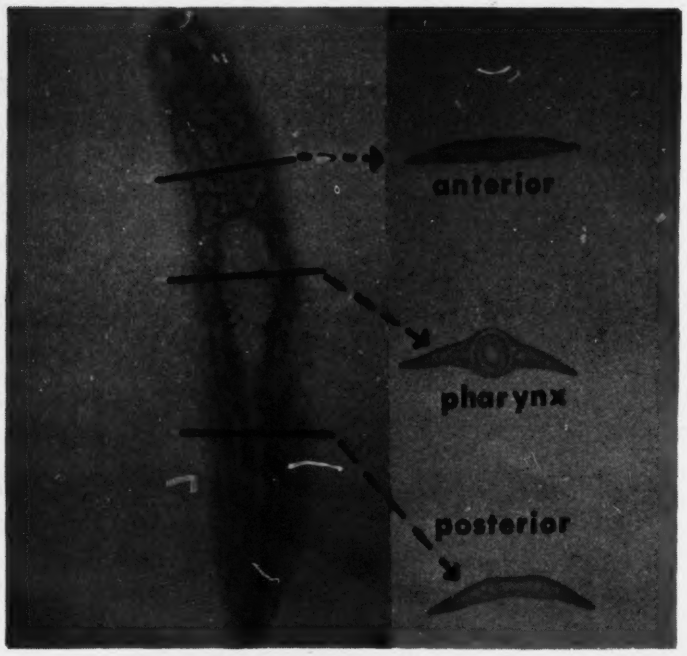


Fig. 7.2
Composite photographs showing regions through which the cross-sections were taken. Dorsal sides of sections are toward the top. Both pictures x20.

3. Cross sections, slide #11. Examine under low and high power. There are at least three sections of planaria on the slide: one through the anterior end, one through the posterior end and one through the pharynx. With some thought, you should be able to tell which one is which. Under high power, look at the single layer of ectoderm cells on the outside of the body, and see that the ectoderm cells on the ventral side are ciliated. Examine the single layer of

endoderm cells that lines all branches of the gut. How do these differ from the ectoderm? The mesoderm fills all the space between the other two layers; made up of connective tissues and muscle cells. Both these elements are long and slender, and though you cannot distinguish individual cells, you can make out the general direction they run. Distinguish a definite layer of circular muscle near the outside of the body; and strands of transverse muscle extending from dorsal to ventral.

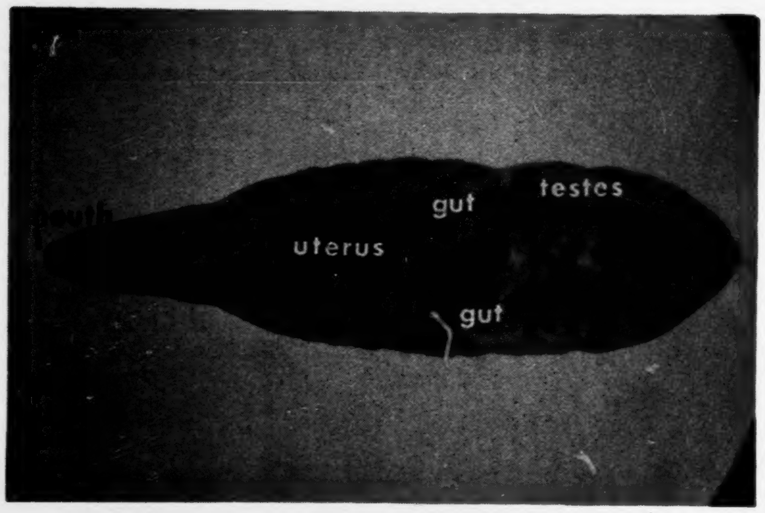
4. Regeneration. Planaria have been used extensively in studies of regenerative powers of animals--small pieces of these worms will often produce whole new animals, under favorable conditions. Formulate a small-scale experiment to demonstrate regeneration; either by yourself or with one or two other students. We can allot each person one or two Syracuse watch glasses and a few planaria, so by pooling resources you should be able to carry out a respectable experiment. Make sure you have a hypothesis or question that will yield meaningful answers. Store the watch glasses stacked or covered, with vaseline between to prevent evaporation of water.

Report #5. Make a clear report of the regeneration of planaria. If you did the work with other students, make only a single report. This is the only report in which joint authorship will be allowed. Indicate the joint authorship alphabetically on the first page of the report. One grade will be assigned to the report, and this grade will be given to each author. This means that you should be completely satisfied yourself with the report before it is turned in, even if you are not the person who made the final draft.

B. A LIVER FLUKE, OPISTHORCHIS SINENSIS (FORMERLY CLONORCHIS). In the planaria, we did not even attempt to study the reproductive system, because it develops from the mesoderm only at certain times of the year, and is invisible at other times. The flukes are in many ways similar to the planaria, but since they are parasitic they have placed greater emphasis on the reproductive system. We study the fluke here, primarily to see this system.

The Chinese liver fluke is widely distributed throughout the world where people eat raw fish and other freshwater crops without adequate cooking. Later you will see why this is true. Humans are its definitive hosts, and in them it produces "liver rot."

Fig. 7.3 Opisthorchis sinensis, x20, from human Liver. A few landmarks are marked, to get you started in examination of the slide.



1. Shape. Examine slide #12 under low power of the compound microscope, and with the dissecting microscope. The worm is a flat, leaf-shaped animal, and in life varies from reddish-brown to grayish-yellow, but in your slide it has been stained to show internal organs better. The anterior end is the narrowest end, and you are looking at the dorsal side of the animal.

2. External features. Find the mouth near the anterior end, surrounded by the anterior sucker disc. Then find the smaller posterior sucker disc, lying 1 or 2 mm. behind the anterior one.

3. Digestive system. A pharynx lies posterior to the mouth, and behind that the gut divides into two branches. There is no anus.

4. Reproductive systems. The animal is hermaphroditic, with both male and female systems within one individual.

   a. Female system. Find the dark-colored, coiled uterus, filled with fertilized eggs, in the region behind the posterior sucker disc. When the eggs leave the body, they pass forward through the genital pore, which you can see just in front of the posterior sucker disc. Behind the uterus, in a row, are the ovary and the seminal receptacle.

   b. Male system. Posterior to the seminal receptacle are two testes, each with finger-like projections that reach nearly to the sides of the body. A nearly-invisible duct carries sperm forward to the genital pore.

   c. When two worms copulate, they come together and attach their genital pores together. Sperm from each enters the other, and the sperm move up through the coils of the uterus, past the ovary, and into the seminal receptacle, where they may live for a long time. As each egg is produced in the ovary, it passes out and past the mouth of the seminal receptacle, where a single sperm will fertilize it. Then the brown, heavy egg shell is secreted around it by a small shell gland. Then the eggs move into the uterus, to develop for a time before they are expelled through the genital pore.

5. Larval development. The worm lays the eggs, which are passed out of the host's body with the host's feces. They go through a complex life cycle before a new human is infected. The following are on demonstration microscopes--get a general idea of what these larval stages look like:

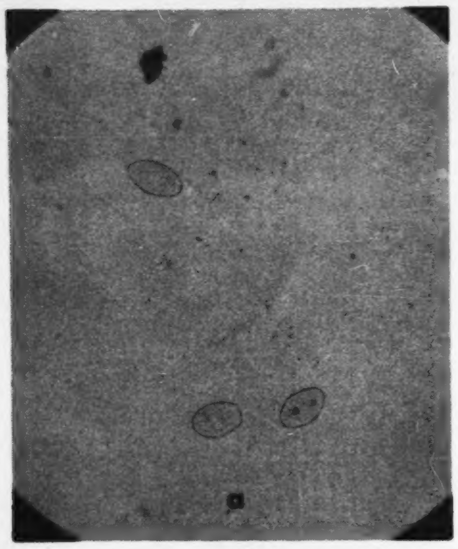


Fig. 7.4 Fluke eggs with miracidia inside.

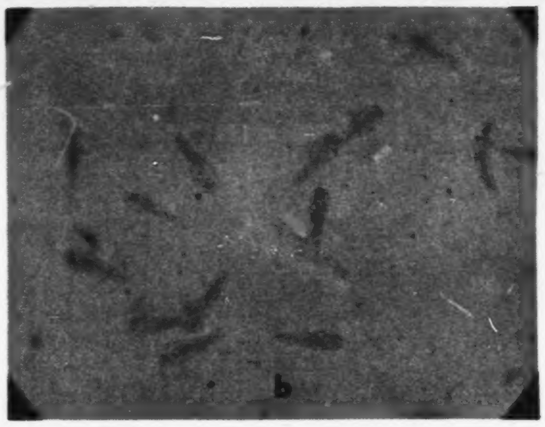


Fig. 7.5 Fluke miracidia in water. They need to penetrate a snail to develop.

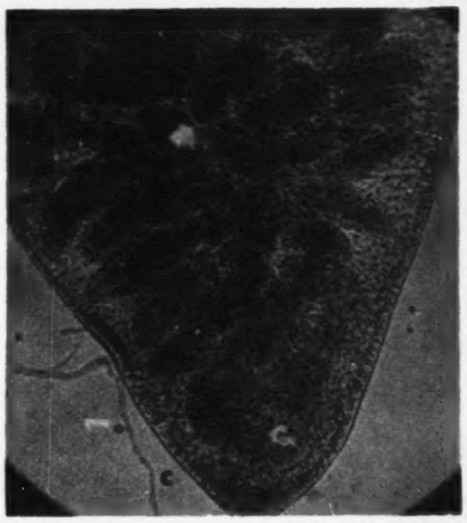


Fig. 7.6 Part of a fluke sporocyst, dissected out of a snail. Note the redia inside.

(All photos x100)

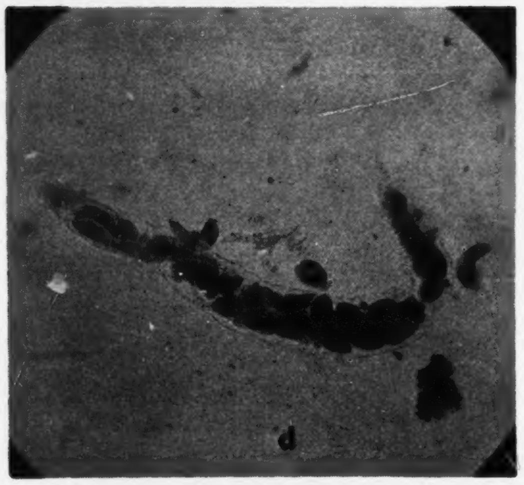


Fig. 7.7 Fluke redia from a snail, with cercaria inside.

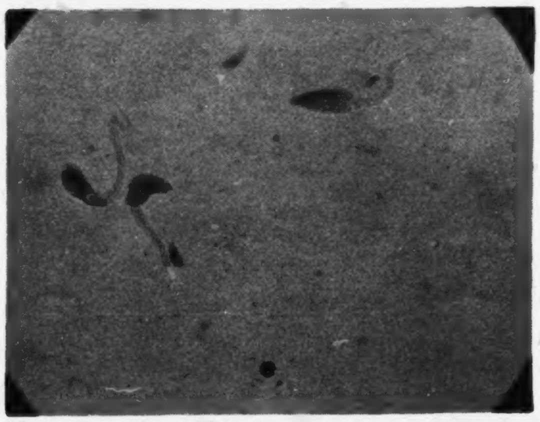


Fig. 7.8 Fluke cercaria in water, released from redia.

a. Eggs. Notice that these are really embryos inside an egg shell. Eggs must be deposited into warm water or they will die. If conditions are favorable, they will hatch into--

b. Miracidia, or tiny ciliated larvae. These will live in water for 8 to 10 hours, but in that time they must find a member of a particular species of water snail. If they do, they will burrow into the snail and find a favorable location, where they will lose all their external features and become--

c. Sporocysts. These grow in the snail tissue, and then by a kind of internal budding (asexual reproduction) each sporocyst will produce a large number of--

d. Redia. These are hardly distinguishable from sporocysts. Again by internal budding, each redia will produce a large number of--

e. Cercaria, or swimming larvae. These break out of the snail and swim in the water. If they find a fish of the right kind, they burrow under the scales and encyst themselves as--

f. Metacercaria. If a person eats the fish raw, the metacercaria will become active and go up the bile duct to the liver, grow, and become--

g. Adults. They may live 15 to 30 years in the liver.

Most flukes have complicated life cycles like this, though the exact pattern varies from species to species. In many flukes, a different particular host is required for each of the larval stages; in others, some stages are dropped from the cycle.

C. A TAPEWORM, TAENIA PISIFORMIS. A tapeworm is not a single individual, but a whole colony of worms, all budded off from the original animal. Each segment reminds you of a fluke, but one in which all digestive apparatus and sense organs have been eliminated. Each segment, then, is a reproductive sac, that absorbs food from the gut of its vertebrate definitive host. Intermediate hosts are variable from species to species, but in general tapeworms require fewer hosts than flukes.

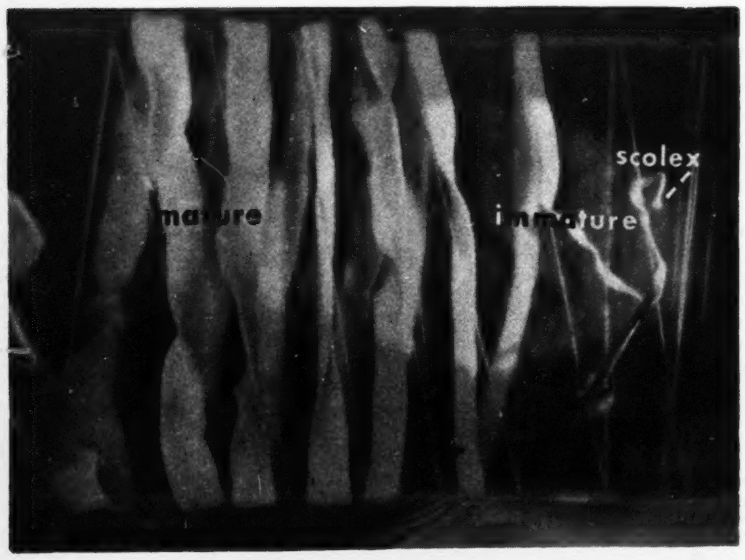


Fig. 7.9
Part of a tapeworm, Life sized. Some of the proglottids at the left are nearly mature, but there are no gravid proglottids here.

1. Shape. Become oriented by examining the photograph above. Note the ribbon-like form, narrow at one end, wide at the other. The narrow end terminates in the scolex, or original individual, which continually buds off new individuals behind itself. Each segment behind the scolex is called a proglottid, and is a separate individual of the colony. Where is the oldest individual in the colony? The next oldest? The youngest? Moving backward from the scolex, you encounter embryonic proglottids, then sexually mature proglottids, then gravid (egg-filled) proglottids. Slide #13 contains several selected pieces, arranged in order. Examine the slide with binocular and compound microscopes, and be sure you can identify the scolex, its suckers and hooks, and the three kinds of proglottids described above.

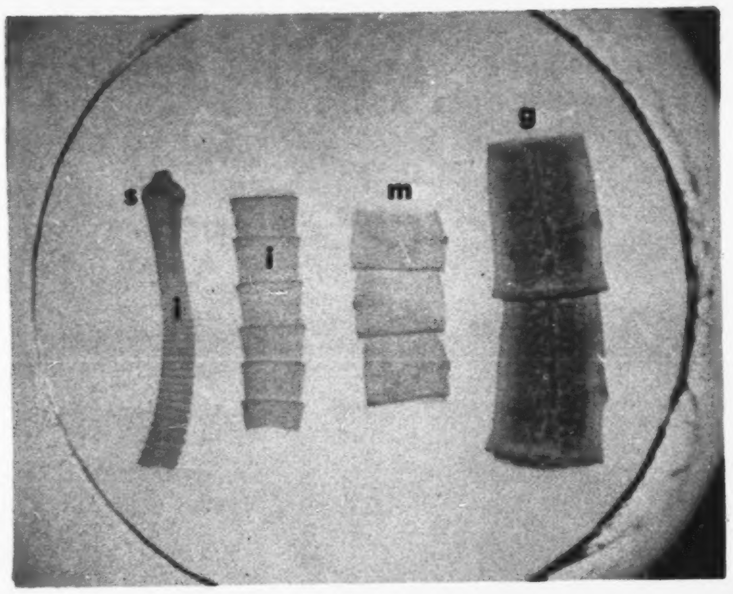


Fig. 7.10 The tapeworm slide, x10. From left to right; scolex with some immature proglottids; more immature proglottids; sexually mature proglottids; gravid proglottids filled with eggs.

2. Reproductive systems. Each proglottid is hermaphroditic, like the fluke, and has much the same organs as the fluke has. Examine a mature proglottid under low power. The genital pore is on the side of the proglottid, and you can identify several parts of the female and male systems. Copulation in tapeworms is much like that of flukes. There is one difference in reproduction: proglottids do not usually lay their eggs; instead the whole proglottid will drop off the end of the colony and leave the host's body with the host's feces. Sometimes, after deposition of the feces, the proglottid will burst and scatter the eggs around.

3. Larval development. If the eggs get into a proper intermediate host, they will hatch and burrow through the gut wall of the host into the blood stream. They will then ride around to a good location, where they will grow and eventually encyst themselves as bladder-worms. Examine the microscopic demonstration of tapeworm bladder-worm larvae, and see where they get that name. If the intermediate host is then eaten by the proper definitive host, the bladder-worm will attach itself to the host's gut wall, and become a scolex.

Fig. 7.11 Bladder-worm larva of tapeworm, x20. The dark inner part becomes the scolex.

II. NEMATODA, THE ROUNDWORMS.

These worms show two definite evolutionary advances above the flatworms: an anus and a pseudocoelom. They also show apparent degeneration: they have few sense organs, and they possess only longitudinal (no circular) muscles. Their position in the evolutionary scale is uncertain. However, they are highly successful animals--in addition to the numerous parasitic species, there are even more species that live in soil, water and decaying vegetation. You will see the main characteristics of the Nematoda, without attempting a detailed study of their anatomy.

A. A VINEGAR EEL, TURBATRIX ACETI.

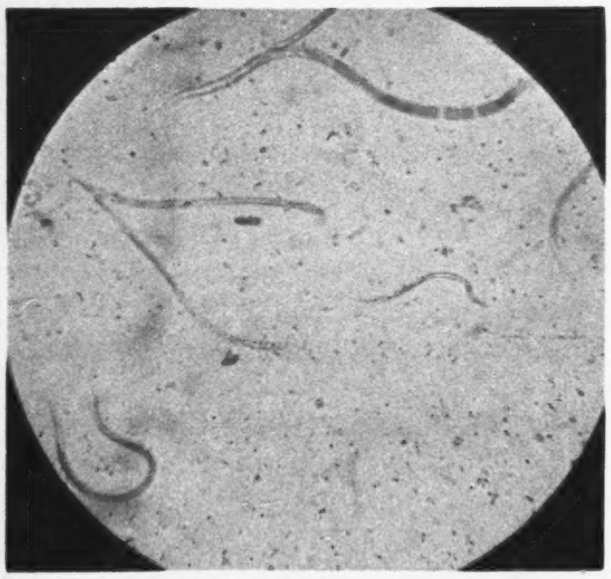


Fig. 7.12
Several specimens of Turbatrix aceti.
Low power.

The culture material provided you is unpasteurized vinegar. The worms eat the bacteria that have made the acetic acid. Take material from the bottom of the culture and examine under low power.

1. Activity. Observe the typical lashing movement of the body. Once you have seen a nematode move, you will never mistake it for anything else. The animal cannot crawl because it has no circular muscles, only longitudinal ones. How is it possible for it to make any progress forward?

2. To see the animals more clearly, add one or two drops of Bouin's solution to the side of the coverglass and let it diffuse inward. These tough animals are hard to kill, but this should at least slow them down. Observe the mouth at the anterior end, the anus near the posterior end, and the one-way, tubular gut that connects them. Most of the rest of the body is filled with reproductive organs.

B. A LARGE PARASITIC NEMATODE, ASCARIS LUMBRICOIDES.

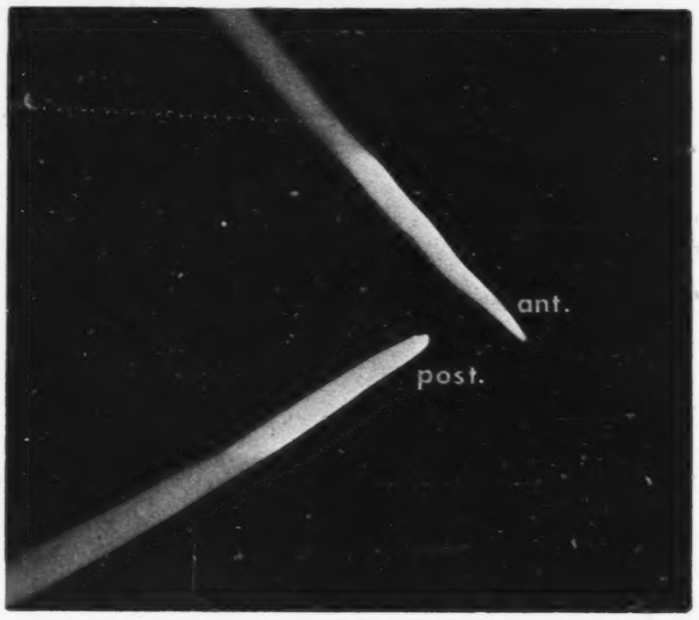


Fig. 7.13 The two ends of a female Ascaris lumbricoides, life sized, to help you distinguish between them.

Dissect a female worm under water, to support the internal organs.

1. External structure. Find the mouth on the narrow anterior end; the anus near the posterior end, and the genital pore, about one-third of the way from anterior to posterior, on the ventral side of the body.

2. Internal structure. Cut the animal open along the dorsal surface, and pin it open. Notice that the internal organs lie in a space, the pseudocoelom. Why could you not have opened a planaria or a fluke in this way? Find the flat, ribbon-like gut that extends from the mouth to the anus. Everything else inside is reproductive organs. If you start at the genital pore, you can follow the single vagina until it divides into two uteri, which are the largest tubes in the body. Each of these connects with a coiled mass of oviducts, the middle-sized tubes; and finally to the small tubes, the ovaries.

3. Cross section, slide #18. Find the body wall, the flat gut, and all the tubes as above. Notice the pseudocoelom, which though apparently empty, is made of huge, vacuolated, thin-walled cells. Also note the feathery arrangement of the muscle fibers. Remember that every fiber runs lengthwise of the body, so you will see a cross-section of each muscle cell, and none in longitudinal section.

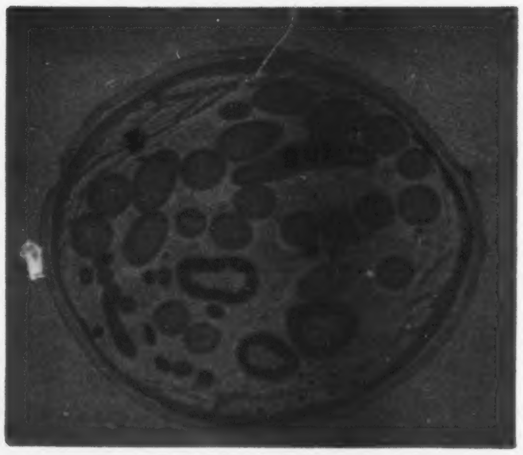


Fig. 7.14
Cross-section through female Ascaris lumbricoides, x20.

LABORATORY WEEK 8:
ANNELIDA AND ARTHROPODA

The Annelida (segmented worms) and the Arthropoda (animals with jointed legs and external skeletons) are so similar to each other in so many ways, that some taxonomists would like to put all representatives of them into one single phylum. The trochophore larvae of the marine forms of each phylum are so much alike that it takes an expert to tell whether one is an Annelid or an Arthropod. Both groups grow beyond this larval stage by budding off new segments from the posterior end; adults of both groups begin as a series of similar segments, and each segment contains a complete set of parts and organs. The similarity between these groups extends even to the way the mesoderm and coelom are formed, and the structure and function of their nervous systems.

The differences between these two phyla lie in the structures of the adults, because evolution has led them in different directions. The work for this week will show you some of these differences.

I. ANNELIDA.

As you observe members of this phylum, look especially for the following:

1. The large number of similar segments that make up the body (homonymy).
2. Specialization of some segments for particular functions (heteronymy).
3. The pair of appendages--parapodia--on most segments, made of two lobes plus a number of stiff bristles--chaetae--supporting the lobes.
4. A true coelom: a space between the body wall and the gut, which makes it possible for digestive functions to be separate from body movement.
5. A well-developed circulatory system, with blood.

A. A CLAMWORM, NEANTHES SP.

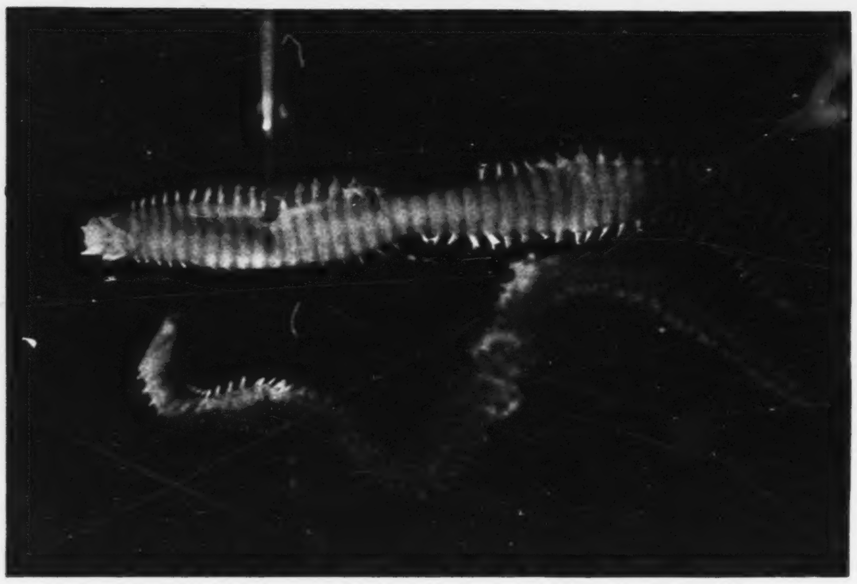


Fig. 8.1
Preserved clamworm,
Neanthes sp., x1.
View from dorsal side,
anterior end above,
posterior end below.

Examine a preserved specimen under water. Do not injure it, but return it intact. We use this worm to show you the general external structure of a typical primitive annelid; internal dissection of them shows very little. This is a member of the class Polychaeta, meaning many-bristled; and it is described as an "errant" worm--it actively swims around in the ocean, rather than sitting quietly in a burrow, as its sedentary tube-worm relatives do.

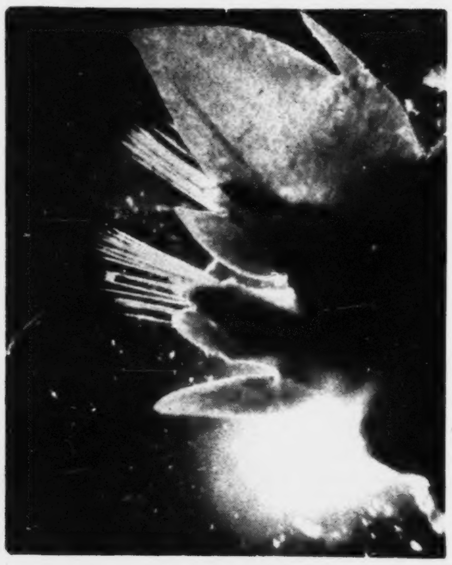


Fig. 8.2
Parapodium of
Neanthes x20.

Examine its parapodia. How many per segment? Are they on all segments? How many lobes on each parapodium? Bend the worm and examine one parapodium with the binocular microscope. Find the chaetae that run through and support the lobes. Slide #19 is of a whole parapodium, cleared (= made translucent) so you can see it better. Examine it for the relationship between the lobes and the chaetae.

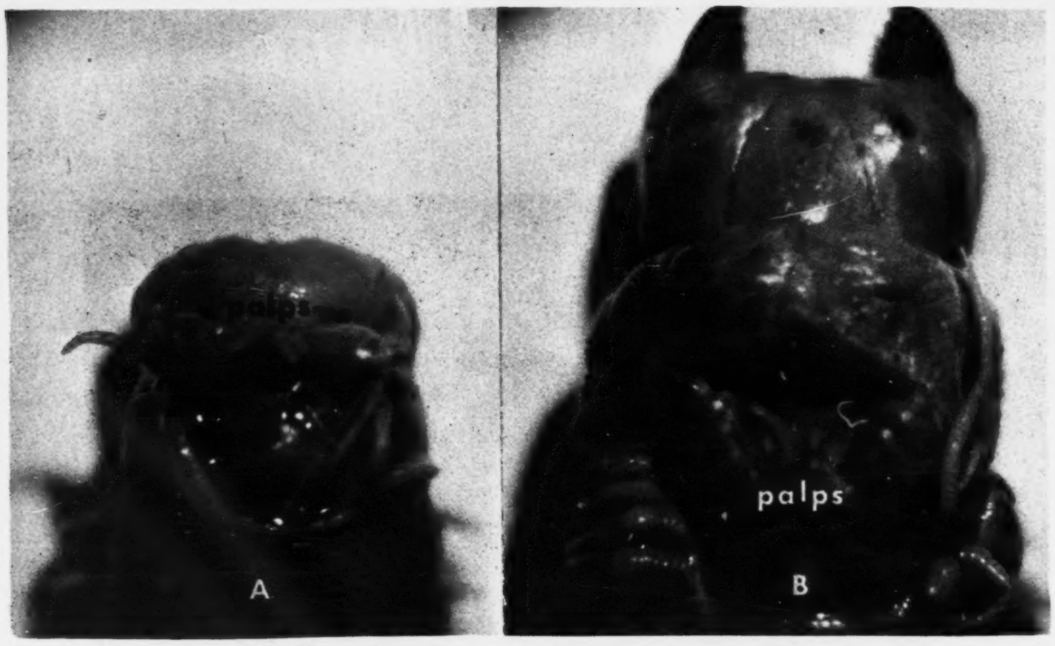


Fig. 8.3
Composite of two anterior ends of Neanthes, dorsal view x10. A with hooks not everted, B with hooks everted.

Neanthes browses on material along shorelines. It ingests food by everting its pharynx through its mouth, and often when killed they die with the pharynx protruding through the mouth. If there is a pair of black hooks on the anterior end of your worm, then these hooks are on the pharynx. Orient yourself by looking at the antero-dorsal side of the worm for a pair of small pad-like projections, the palps, which are on a half-segment called the prostomium. If the pharynx is not everted, then these palps are the most anterior part of the worm. Refer to the illustration for help in finding the palps. When you have found them, then look carefully at the prostomium and determine its size and boundaries. Look also for any tentacles on the prostomium. Under the binocular microscope, you should be able to find two pair of very tiny light-receptive eyes.

The first complete segment of the body (counted as segment #1 when counting segments) is the peristomium, which lies just posterior to the prostomium. What extra structures are found on this segment? If pro- means before; peri- means around; and -stomium means mouth; then what do you think about the appropriateness of the names for these two anterior segments?

Segments posterior to the peristomium are merely numbered, with the peristomium being assigned segment #1. Compare the number of segments found in your worm with the number in worms studied by your colleagues in the laboratory. What do you conclude about the regularity of numbers of segments? Which segments do not have parapodia? Is this a regular situation in other Neanthes?

Find the anus at the posterior end of the worm. What is its location? Are there any extra structures on its segment, or on other segments at the posterior end of the worm?

As far as you can observe on the outside of the Neanthes, would you say that this worm is mostly homonymous or mostly heteronymous?

B. AN EARTHWORM, LUMBRICUS TERRESTRIS.

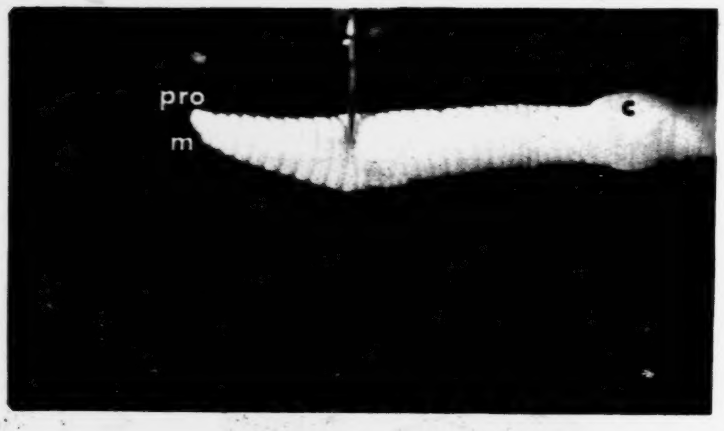


Fig. 8.4
Lateral view of
Lumbricus, x1.
pro= prostomium,
m= mouth,
c= clitellum.

Most people think of the earthworm as "the typical" Annelid, probably because it is so common in our area. As a matter of fact, however, it is not at all typical of Annelids in general. It has become highly specialized for subterranean life, and in making these adaptations it has lost all its parapodia except its chaetae--and even these are reduced in size and numbers. The few chaetae give this worm its class name, Oligochaeta, where oligo- means "few."

Put an earthworm into a dissecting pan with water. Since you will be dissecting this worm, plan to discard all parts when finished.

1. Segmentation. This animal does not evert its pharynx through the mouth, so the most anterior portion you will see is the prostomium, which occupies the same relative position as it does in Neanthes. Behind it is segment #1, the peristomium. In further work on Lumbricus, make sure you know how many segments there are, and in or on what segments the specialized organs are found. Compare your findings with your colleagues, to see what kind of regularity and diversity is found in this species. There are few special structures on the outside of the animal. Can you find palps, eyes, tentacles? Where do you find the anus? Note that there are no parapodia, but there are chaetae. Examine under the binocular, and determine their placement and arrangement. Is it regular from segment to segment?

2. Locomotion. Carefully observe a living earthworm as it crawls. Describe as clearly as possible, how it moves, how the muscles must behave in order to bring it about, and what the chaetae do.

3. Reproduction. Marine Annelids like Neanthes can shed their eggs and sperm directly into the water, but this would be impossible in freshwater or land forms. Do you see why? Lumbricus has evolved a complicated system of reproduction, which provides an artificial pool of water in which the embryos develop. The process begins when two worms come together, and each transfers sperm into a seminal receptacle of the other. The two worms separate, and the worms produce eggs to be fertilized by those sperm.

Find the saddle-like clitellum which covers the dorsal sides of several segments. When the eggs are mature, a coccoon loosens from the clitellum that has produced it, and it slips like a ring toward the anterior end of the worm. On its way, it collects eggs, then sperm, then slips entirely off the worm, the ends close, and it provides a sac in which the embryos can develop. With the binocular, find the following paired genital openings, all located more-or-less ventrally:

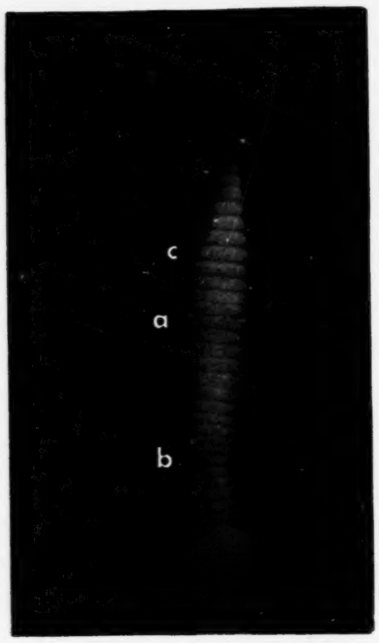


Fig. 8.5
Ventral view of
Lumbricus, x1.
a, b and c as
on opposite page.

a. The largest, obviously lipped, are the *openings of the sperm ducts*.

b. Eleven segments back are the *openings of the oviducts*.

c. Count five segments forward from *a*, and on that segment look in the grooves between the segments both in front and behind it for the tiny *openings of the seminal receptacles*.

Try putting two worms together in such a way that the openings of the sperm ducts of each one are in contact with the ventral side of the clitellum of the other one. This is the way the worms look when they mate. Sperm ejected from the sperm ducts travel via a pair of *grooves* to the seminal receptacles of the mate.

With a sharp pair of scissors, working under water, make an incision through the body wall of the worm, on the dorsal side slightly to one side of the midline. Open the worm and pin it back, all the way from the prostomium to the clitellum. Move internal parts very gently, because they are brittle.

Knowing where the external openings of the reproductive organs are, you should be able to find the organs themselves without much difficulty: the *testes*, the *ovaries*, and the *seminal receptacles*. Do so, but do not be concerned if their precise location is not perfectly clear to you. By comparing with the findings of others, how much regularity do you find in their location and extent?

4. The *coelom*. When you opened the worm, you cut through the body wall into a fluid-filled, compartmented space, in which the reproductive organs were located. The *gut* runs through this space (though as you will see later it is not technically inside the coelom), but little else is found in it. With careful examination under the binocular microscope find the inner ends of the *chaetae* and the muscles that control them; also find some tiny, knob-shaped, ventrally-located *nephridia* in each segment. Notice that the coelom is divided into compartments by transparent *septa*. How do these compare in location with the external segmentation of the worm? Also notice that the gut is more or less free from dependence on motion of the body wall for moving food along. Make an incision into the gut in several places, and see that the wall is thick and muscular, not thin as in *Ascaris*. Do you see why the Annelids are described as having a "tube within a tube"?

5. Digestion. Annelids have an anus, which means that food moves in only one direction through the gut, and may be treated differently in succeeding parts of the gut. This improved "dis-assembly line" is much more efficient than the system in flatworms, and the musculature present makes motion through the gut more controlled than the situation in the Nematodes, which have a one-way gut but little control over movement through it.

Find the following parts of the gut, and try to decide what happens to the food in each: The *mouth*, the muscular *pharynx*, the narrow *esophagus*, the sac-like *crop*, the muscular *gizzard*, and the long straight *intestine* leading to the *anus*. Does it appear that there are any *digestive glands* that might secrete enzymes into the gut? What would the presence of such glands tell you about intracellular vs. extracellular digestion of food? What would their absence tell you? (Think carefully here--it may fool you.)

6. Circulation. In most Annelids, blood never escapes from blood vessels--that is, they have a closed system. The most obvious parts of this system in Lumbricus are:

a. The dorsal vessel, usually still attached to the dorsal side of the gut.

b. The five pairs of commissures ("hearts"), around the esophagus, which carry blood from the dorsal vessel ventrally, and connect to--

c. The ventral vessel (or more than one), smaller than the dorsal vessel.

Blood flows anteriorly in the dorsal vessel and posteriorly in the ventral vessel. Many connections are found between the ventral vessel and the gut--blood flows through a sinus in the wall of the intestine, then up to the dorsal vessel. Most of the force for circulation of blood is provided by waves of contraction of the dorsal vessel. The commissures seem merely to equalize pressure throughout the system. (Note: in Arthropods, a homologue of the Annelid dorsal vessel is referred to as the "heart".)

Watch the blood in a living worm, especially in the dorsal vessel. You can observe the contractions in this vessel. They look like peristaltic waves.

7. Nervous system. Find the brain, a bilateral two-part structure which lies on the dorsal side of the anterior part of the pharynx. A number of nerves extend out from it. Two are especially large, the circumesophageal connectives, one of which extends around each side of the pharynx and connect the brain with the large ventral nerve cord. This arrangement of the nervous system is the standard pattern, found not only in Annelids and Arthropods, but with minor variations in all animal phyla from Platyhelminthes to Molluscs. Only in Echinoderms and Chordates will you find any different standard pattern.

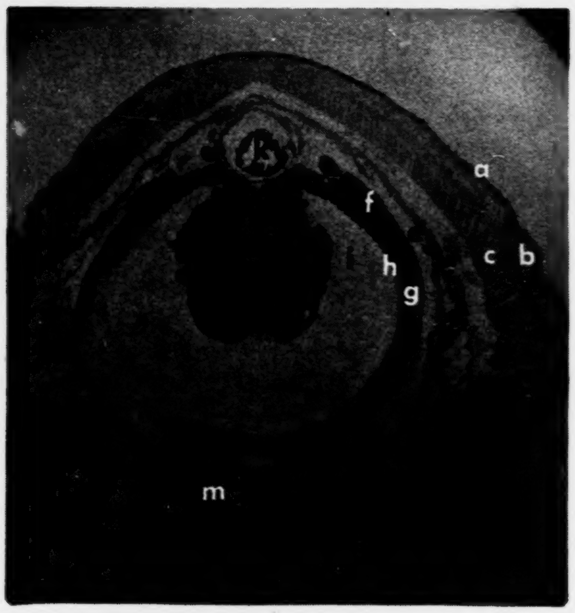


Fig. 8.6
Cross-section of Lumbricus, x20. Letters of labels correspond to letters on opposite page except d, which is too thin to show in this particular section.

8. Cross section, slide #14. Apply what you have learned above to a slide of a cross-section through the intestinal region of Lumbricus. Orient yourself by studying it from outside to inside.

a. Hypodermis, a single layer (purple) of columnar cells, which has secreted a very thin cuticle on the outside of the worm. This is the only ectodermal derivative in the slide; everything from here to the mucosa (h below) is derived from the mesoderm.

b. Circular muscle, the muscle cells cut in longitudinal section (pink).

c. Longitudinal muscle, the muscle cells cut in cross section (pink). These look like the longitudinal muscle in Ascaris, in their arrangement.

d. Parietal peritoneum, purple cuboidal cells lining the inside of c.

e. Coelom, empty space, variable in thickness.

f. Chlorogogue layer, derived from the coelom's other edge, the visceral peritoneum, but here tremendously overgrown. It looks like a jumble of blood vessels, glandular tissue, ducts and empty spaces. It looks that way because that is what it is. It is difficult to see where the coelomic inclusions end and this layer begins; but the inner edge of the chlorogogue layer is sharply set off from the muscle, and is stained a different color as well.

g. Visceral muscle (thin and pink). Separate layers of muscle are not clearly set off from each other.

h. Mucosa, very tall columnar cells (purple), involved in digestion. These are of endodermal origin.

i. Lumen, or empty space, of the gut.

After you have studied the above, you should be able to distinguish the following special structures:

j. Typholsole, a fold of mucosa and other tissue that hangs down into the gut from its dorsal side.

k. Dorsal vessel, just dorsal to the typhlosole.

l. Ventral nerve cord, in the coelom, on the ventral side.

m. Ventral vessel or vessels, very close to the nerve cord.

The coelom in your particular slide may have parts of various organs in it--chaetae, muscles of the chaetae, nephridia, etc.

II. ARTHROPODA.

We will make no internal dissection of any Arthropod in this course, but will confine our work to demonstrating the following:

1. The great heteronymy of adults of this phylum, which has arisen from homonymous larvae. We will see fusion of many segments, and increase or decrease in sizes of appendages. Most of this will be done by observation of crayfish appendages.

2. The structure of an insect, said to be the most successful body plan in the world.

A. A CRAYFISH, CAMBARUS SP.

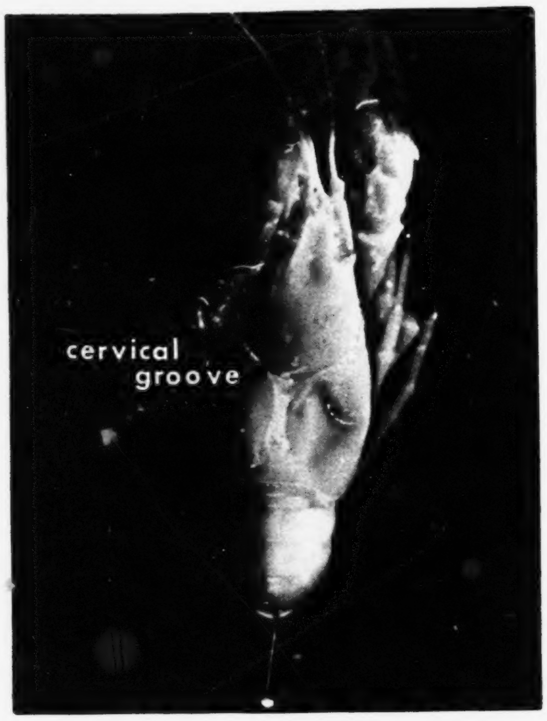


Fig. 8.7
Cambarus sp., x½.

The crayfish is a large member of the Class Crustacea, and therefore is related to the tiny crustacea that you saw in your water sample in large numbers. We study a large one because its parts are easier to see.

Put a crayfish into a dissecting pan, with water. It is not so important to dissect under water with this animal as it was with the earthworm, because we will not be dissecting the soft inner parts (except the gills) and the outside supports itself with its exoskeleton. Be sure to discard all parts when you are finished.

Notice that the whole animal is covered by an exoskeleton made of chitin, and this serves both in support and protection. The exoskeleton must shed, by a process of molting, before the animal can grow in size. After a molt, the chitin (which corresponds to the cuticle of Annelids) is soft, but it becomes hardened by deposits of salts in a short time. The parts of the crayfish that you can see are the body and its appendages.

1. Divisions of the body. It has two well-marked parts, the cephalothorax anteriorly and the abdomen posteriorly. Which of these looks to be the more homonymous?

   a. Cephalothorax: The carapace is the chitinous covering of the whole structure, and it ends anteriorly in a dorsal projection, the rostrum. A cervical groove separates the head from the thorax. On the head identify the short antennules, the long antennae, the stalked compound eyes, and the ventrally-placed mouth covered with mouth parts, to be studied in 3 below.

The carapace of the thorax is divided by two grooves into a median region and two gill regions laterally. Notice that the ventral edge of the carapace is free; lift it and see the gills in the gill chamber.

b. Abdomen: This part of the body consists of six similar segments and, at the posterior end, the telson or tail-piece. This telson plus the paired, fan-shaped uropods make up the tail fin. Find the anal opening on the ventral surface of the telson. How is the first abdominal segment different from the other five? Note that the segments are joined together by soft membranous areas in such a way as to permit movement between them.

2. A typical segment with its appendages. Make a careful vertical cut through the abdomen, so that you cut apart the third and fourth abdominal segments. Look at the cut surface of the fourth segment. We choose this one because it is probably the most primitive--that is, most like the segments of the larva that gave rise to the heteronymous adult segments.

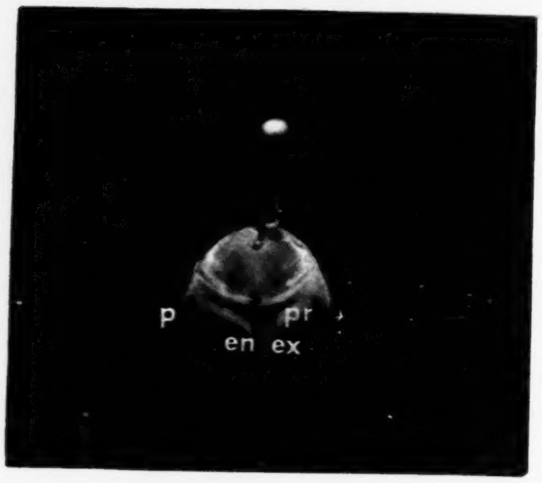


Fig. 8.8 Posterior view of the cut end of abdominal segment 4 of Cambarus, x1.
t= tergum, s= sternum, p= left pleuron.
The right appendage is labeled:
pr=protopodite, ex= exopodite,
en= endopodite.

a. Body. The segment consists of a dorsal arched portion, the tergum; a ventral flat portion, the sternum, and lateral projecting portions, the pleura. The large bulk of the segment is made up of muscle, used in bending the tail fin downward. Which parts of the segment are derived from ectoderm, mesoderm, endoderm? The intestine runs as a straight tube on the dorsal side of the abdomen.

b. Appendages. Each of the paired appendages is made up of a two-jointed basal portion, the protopodite; and two distal branches--the exopodite (laterally-placed) and the endopodite (medially-placed). This two-branched condition is called "biramous." It is thought that this is a primitive appendage, and that all other appendages are derivable from this basic plan. Compare this basic plan with that of the Annelid appendages.

3. Serial homology as demonstrated by the crayfish appendages. All appendages are alike in the larva, but they become specialized for various functions in the adult. Notice which parts of the appendages are increased in size and which are degenerate or absent. Whenever a uniramous appendage is derived from a biramous one, it is always the exopodite that is lost.

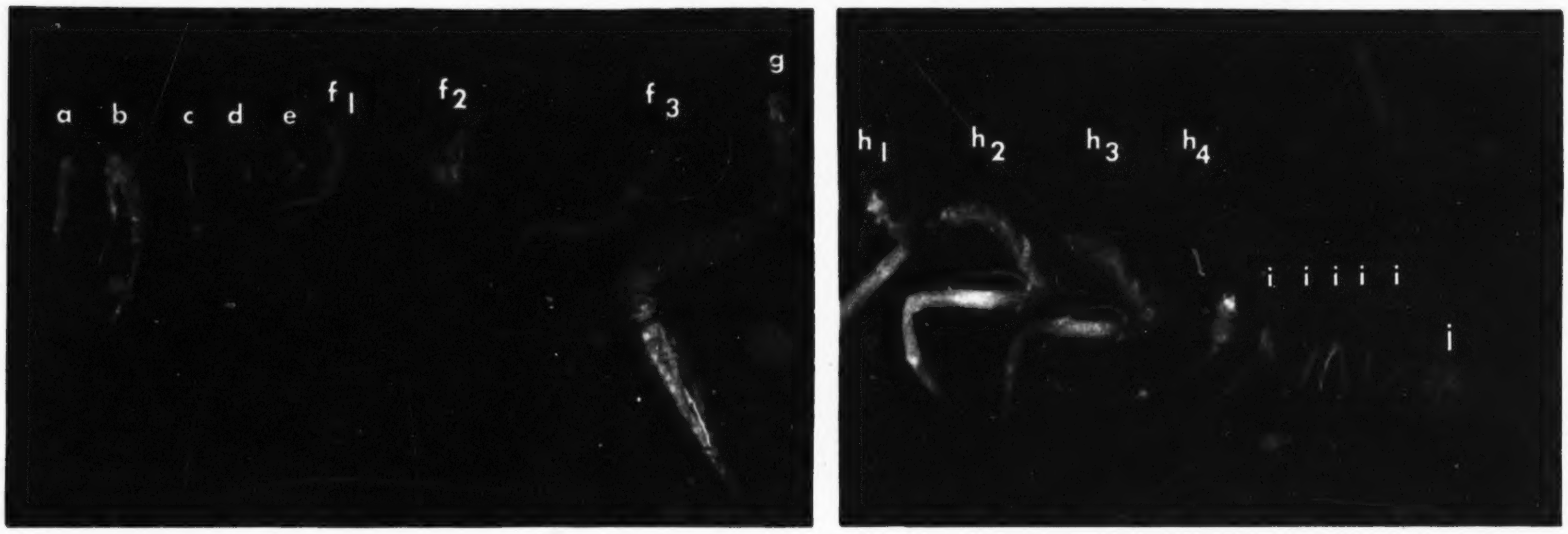


Fig. 8.9 Appendages of Cambarus, x1, to show relative sizes. Labels as below.

First remove the carapace from one side of the animal, exposing the entire gill chamber. Take hold of each appendage in turn, beginning at the anterior end of the animal, and remove it gently but firmly. Make sure you get the whole appendage. Leave the appendages in place on the other side of the animal, for later reference. Lay the removed ones out in order. Some of the appendages have gills or gill bailers attached to them, and these should come out with the appendages. From anterior to posterior, they are:

a. Antennule: short, anterior.

b. Antenna: long, filamentous.

c. Mandible: hard, small, toothlike, on each side of the mouth.

d. First maxilla: very small and transparent, just behind the mouth.

e. Second maxilla: some what larger than the first, but still very small.

f. First, second and third maxillipeds: larger than the maxillae, found arranged one behind the other.

g. Cheliped: the pincers. This may look biramous, but it is not.

h. Four pairs of walking legs, all much alike.

i. Five pairs of swimmerets on the abdomen. All much alike except the second pair in the male, which are enlarged, used for sperm transfer.

j. Uropod: flat, one on each side of the telson.

B. A GRASSHOPPER, ROMALEA MICROPTERA (FLORIDA LUBBER GRASSHOPPER).

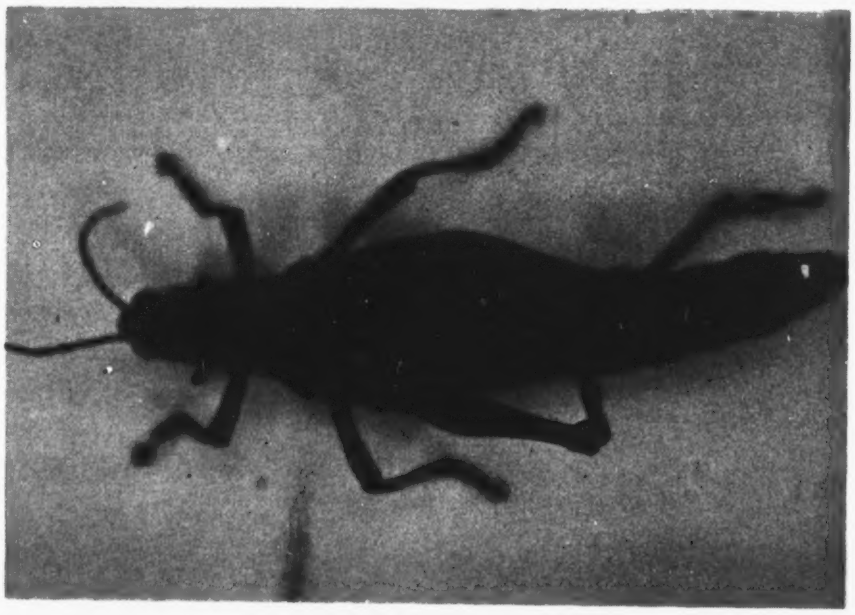


Fig. 8.10
Romalea microptera, dorsal, x1.

This is a large member of the Class Insecta, the most successful class of animals in the world. You can carry the terminology for this insect over into most other groups of insects. This body plan is clearly derivable from something like the Crustacea, but here you will see even greater heteronomy.

1. General body shape. Observe the three main divisions of the insect:

   a. Head.

   b. Thorax, the part that bears the wings and the legs.

   c. Abdomen.

In which of these can you observe segmentation? Observe the chitinous exoskeleton.

2. Head. Will it swivel on the thorax? Note that some of its parts are movable, but others are not.

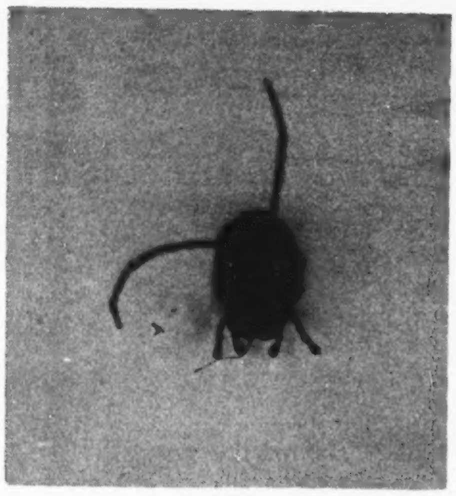


Fig. 8.11
Head of *Romalea microptera*, anterior view, x1.

a. Eyes. Two kinds: the large, brown, oval compound eyes, and the three ocelli, or simple eyes. Ocelli lie in depressions, one at the base of each antenna and one in the mid-front of the head.

b. Antennae. Notice the absence of antennules. Each antenna has a base and a segmented filament. Antennae are functional in smell and/or taste.

c. Mouth parts. The grasshopper has "chewing" mouth parts. Some other insects have mouth parts drawn out into a tube, and are called "sucking" mouth parts. To study the mouth parts best, remove each one with forceps and lay out for observation with a binocular microscope. Find:

1' Labrum or movable upper lip.

2' Labium or lower lip. It has a pair of palps attached to it.

3' Mandibles, black, tooth-like, on each side of the mouth.

4' Maxillae, posterior to the mandibles, each bearing a palp.

5' Hypopharynx, or "tongue," exactly between the maxillae.

3. Thorax. This is made of three segments. Check the attachment of the appendages, and see that the anterior segment has a pair of legs but no wings, while the middle and posterior segments each have both a pair of legs and a pair of wings.

a. Wings. Remove both wings from one side of the grasshopper, spread them out and pin them down. Do they match each other in size and texture? What do you suppose is the function of each wing? Wings are made entirely of chitin, and are not living structures. They are supported by air-filled veins. There are no muscles in the wings as there are in the legs: the muscles that move the wings are inside the thorax.

b. Legs. No adult insect legs are biramous, though their embryos sometimes have biramous appendages. All insect legs have the same general regions, and since these regions are important in classifying most insects, the parts are listed here. Study the jumping leg of your grasshopper, since it is largest and easiest to see:

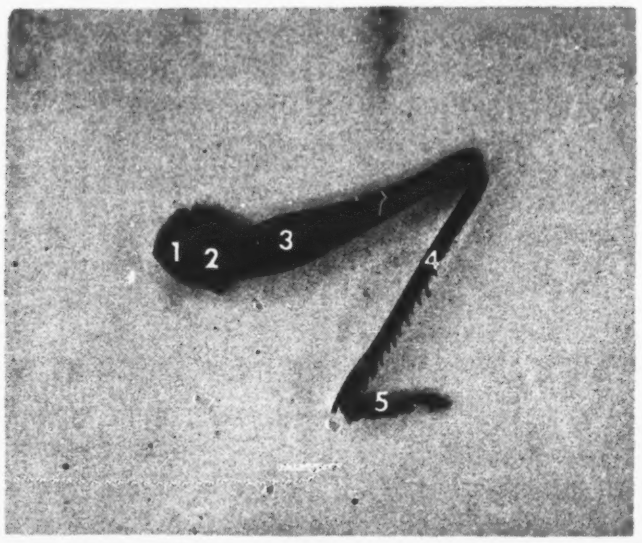


Fig. 8.12
Jumping leg of Romalea
microptera x1. Labels
as below.

1' Coxa, large, round, nearest the body.

2' Trochanter, the next division, somewhat fused with--

3' Femur, large, strong, first long segment.

4' Tibia, long, slender, with several spines.

5' Tarsus, 3-part, the first with three pads, the second with one pad, and the third with two claws and one disc.

c. Spiracles. Find a hole between the first and second thoracic segments, laterally. This is a spiracle or air opening. You will find another one between the second and third segments. Since insects have adopted this method for supplying oxygen to the various tissues directly (air is carried in tracheal tubes throughout the body), you would expect to find many of these spiracles on the anterior, most active part of the body--but you will not find any. These are all there are on the thorax, as well. The abdomen, with the least activity, has the most spiracles: you will find a pair on each of the abdominal segments.

4. Abdomen. Find the following:

a. Ear. It is in a depressed crescent-shaped area just dorsal to the point of attachment of the coxa of the jumping leg. It is flat and covered with a semi-transparent membrane. This is the tympanic membrane of the ear, and is situated on the first abdominal segment.

b. Spiracles. The first abdominal spiracle is just anterior to the middle of the tympanic membrane. It is a slit bounded by lip-like walls. Find the spiracles on the other abdominal segments.

c. Count the segments of the abdomen. There are 7 or 8 complete segments, plus several partial ones called anal appendages near the posterior end of the abdomen. On any one of the abdominal segments, find the tergum and the sternum. The tergum corresponds to a fused tergum-pleuron of the crayfish. What value do you see in having the tergum and sternum attached together by flexible membranes?

LABORATORY WEEK 9:
MOLLUSCA, ECHINODERMATA AND PRIMITIVE CHORDATA

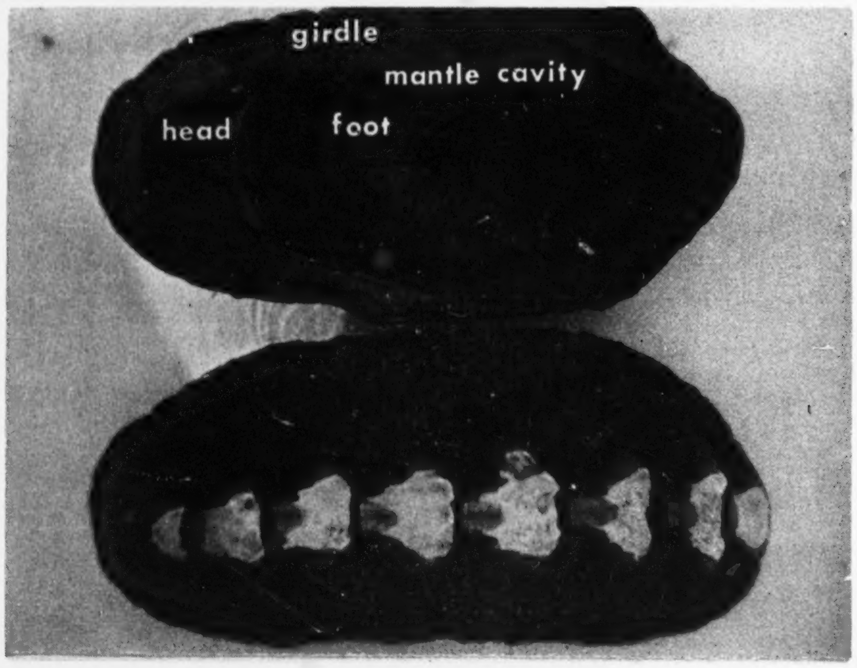
This week's work takes a last look at the Annelid-Arthropod-Mollusc line of development, and introduces the Echinoderm-Chordate line. The animals studied are highly different from each other; about all they have in common is that they are all aquatic, and most of them are marine.

I. MOLLUSCA.

This is a very diverse phylum, containing such apparently different forms as the snail, oyster and octopus. They all have some things in common, however. Their larvae form the mesoderm from pole cells in the same way that Annelids and Arthropods do, and their coelom develops from hollowing-out of blocks of the mesoderm. Their adults are widely different from the Annelids and Arthropods, and the divergence begins early in larval development. All Molluscs begin life with certain body parts in common: a head, a foot, a visceral mass, a mantle, and usually a shell secreted by the ectoderm of the mantle. Molluscs never show any segmentation, even as embryos. As you study the representatives of the Molluscs, make sure you know where the structures are that are underlined above, and in addition know where the mantle cavity and gills are.

A. A GENERALIZED MOLLUSC, CHITON. CLASS AMPHINEURA.

Fig. 9.1 Chiton, ventral and dorsal views, x1.



Chitons are rather rare, browsing creatures of the tidal shores. We study them because they are as close as any living things to the hypothetical generalized ancestors of the molluscs. Examine a preserved specimen, and return it intact without dissecting it.

The dorsal surface of Chiton bears a linear series of eight shell plates. Notice, however, that the animal is not truly segmented. Around the perimeter of the shell area you find the fleshy girdle, which is a part of the mantle. Can you see any evidence of the animal's head from the dorsal side? How can you tell one end from the other?

Turn the animal over and look for the head on the anterior end, separated from the large fleshy foot by an inconspicuous groove. Most of the ventral surface is occupied by the foot. Find the mouth in the head, and probe to find the tongue-like radula lying inside. The radula is armed with tiny sharp barbs, and the whole organ is used like a file, to rasp off pieces of whatever the animal is eating.

There is a deep cleft between the foot and the girdle, called the mantle cavity. Pull the foot and girdle apart and find the gills, small and feathery, in the cavity. Do you find gills in all parts of the mantle cavity?

Make an antero-posterior incision in the midline of the foot, if it has not already been done for you. You can see the coelomic cavity through the incision, and the internal organs, especially the highly-coiled gut. Observe the thickness of the foot. Of what is it mainly composed?

B. ACTIVITY OF A LIVING SNAIL. CLASS GASTROPODA.

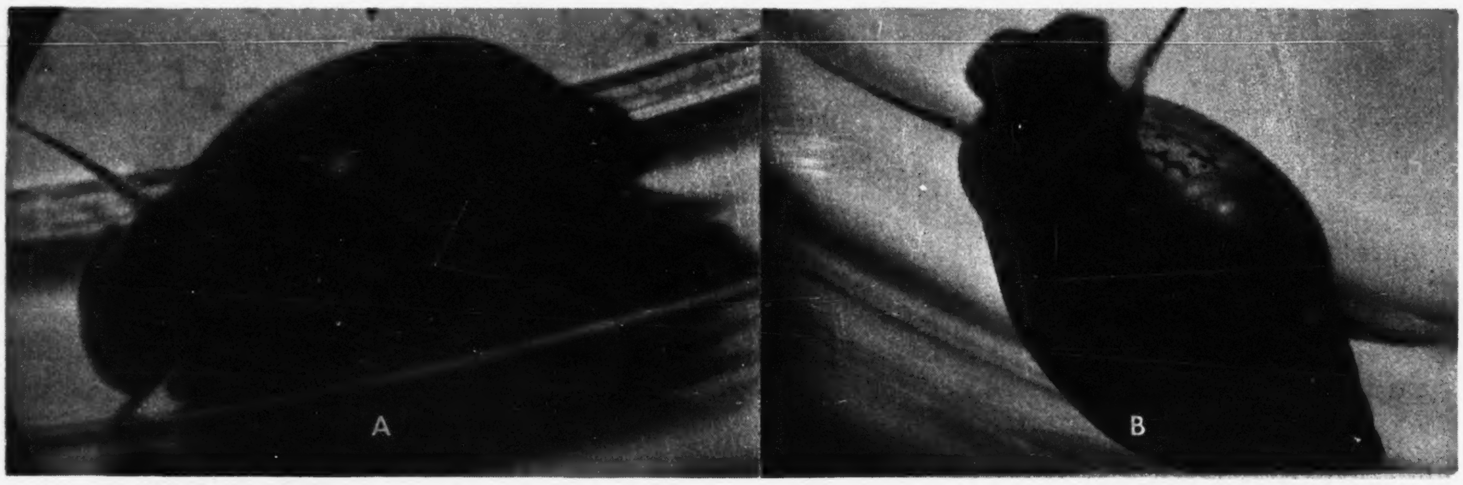


Fig. 9.2 Two views of living snail, x20. A from dorsal, B from ventral, sides.

Snails resemble Chiton in general structure. Observe, in the living specimens in the aquarium, the following differences:

a. The mantle does not cover the head and body except when the animal is disturbed and retracts into the shell.

b. The head is large, not reduced in size, and is provided with a pair of palps. Why could these palps not be called antennae?

c. The shell is not divided into shell plates, but is one solid piece.

d. The body has become twisted to one side, and coiled up. Evidence of this is seen in the twisted character of the shell, which has been secreted by the twisted mantle.

Watch the ventral side of a snail as it walks on glass, using any optical device you can to magnify it. How does the snail move itself forward? Watch the ventral side of the head, find the mouth, and observe the radula as it rhythmically pushes out and into the mouth. What is the snail doing to the glass?

See the demonstrations of various snail shells, on display in the hall case.

C. A MOLLUSC MODIFIED TO STRAIN FOOD FROM WATER, A CLAM. CLASS PELECYPODA.

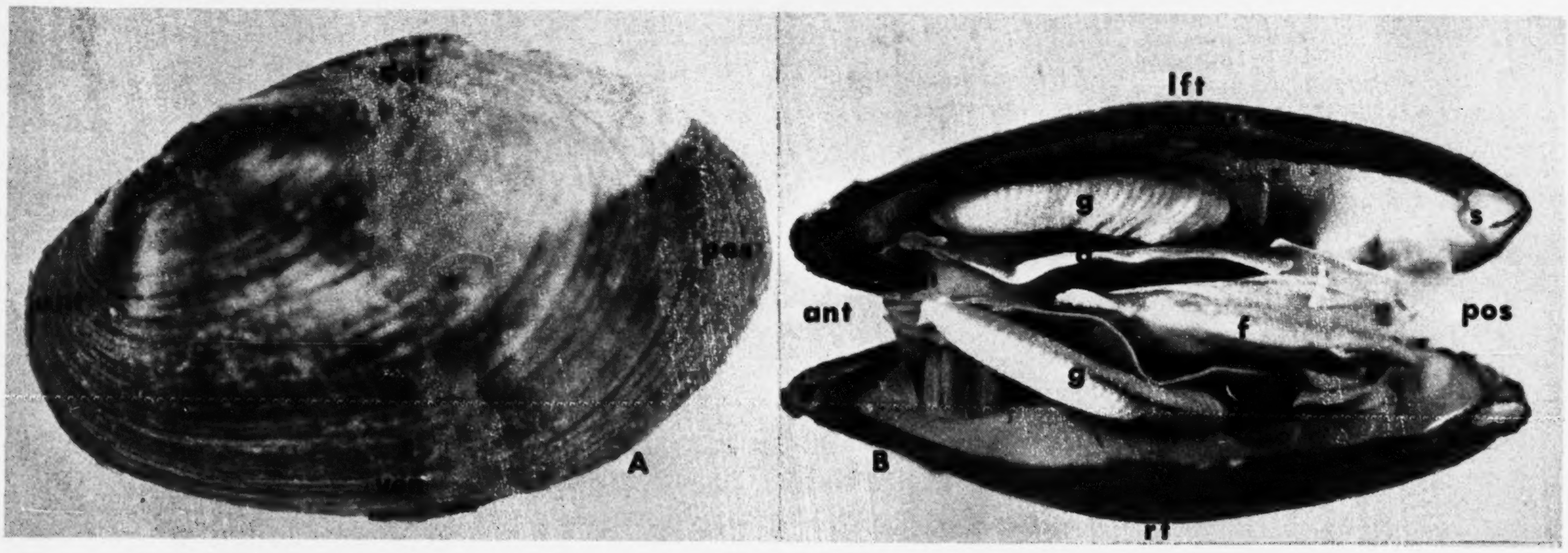


Fig. 9.3 Two views of clam, x½. A from left side, B from ventral side, opened. Labels: anterior, posterior, dorsal, ventral, gills, mouth, foot, siphons.

1. External. The shell is made up of two parts or valves, and the animal is therefore called a "bivalve." The two halves of the shell are fastened together along the dorsal surface by a hinge. The lines of growth of the shell radiate out concentrically from the umbo, which is the oldest part of each valve. (If the shell is non-living, how can it grow?) The more pointed end of the animal is the posterior end. You should be able to see parts of the mantle projecting out of the shell at the posterior end, and these parts of the mantle make up the siphons through which water passes into and out from the mantle cavity. The more dorsal is the exhalent siphon and the more ventral is the inhalent siphon. By examination of the shell you should be able to tell what part of the animal was buried in sand or mud, and therefore what the natural posture of the animal is. Pry the shell partly open, find the foot, and decide how the foot is used in locomotion of the clam.

2. Internal. Lay the animal on one side and dissect away the shell and mantle on the side toward you. Which side of the animal are you dissecting away? With the mantle removed, the two ranks of fleshy gills nearly cover the rest of the animal. You will find two more ranks on the other side of the body also. These will be more visible if examined under water. The gills are adapted to straining food particles from the water. Microscopic cilia on the gills beat and move food particles anteriorly toward the mouth. Notice the absence of a head. Long labial palps nearly surround the mouth and help gather the food in. Compare the clam with a labeled model of a clam dissected, to get an idea of the internal anatomy. Find the anus at the posterior end of the body, near the exhalent siphon. The most important thing to remember about the clam is the route water takes through the mantle cavity, and the route food takes from the time it is taken in with the water until it is expelled from the shell.

D. A PREDATORY SWIMMING MOLLUSC, A SQUID. CLASS CEPHALOPODA.

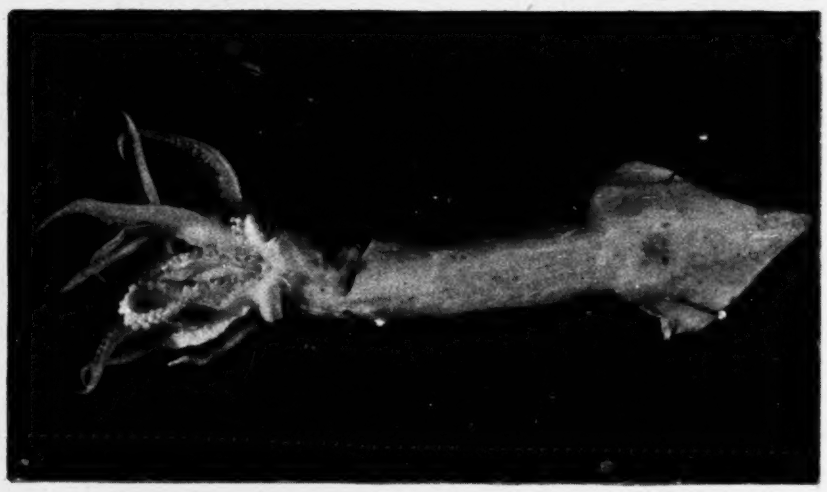


Fig. 9.4
Squid, x¼, as seen from the functional dorsal side. Head and foot to the left, apex of the cone to the right.

1. External. Find the following:

a. Mantle; smooth, cone-shaped, with a pair of fins extending laterally, one on each side, and pulled up tightly around the rest of the body by a collar. Notice that there is no shell visible: the shell is secreted inside the mantle and will be seen later. Notice the difference in pigmentation between dorsal and ventral sides. The animal swims with the apex of the cone of the mantle as its most posterior.

b. Head and foot. These two structures are fused together, giving this group its class name, Cephalopoda. (cephalo- = head, -pod = foot.) The eyes are laterally-placed, the mouth is in the middle of the appendages. Can you find anything that might be used to tear food apart? The appendages are of two kinds: shorter arms and longer tentacles. How many of each? Is this a regular number from squid to squid? Notice the difference in distribution of the sucker discs on each kind of appendage. What do you think is the major function of the appendages? The animal swims with its tentacles as its most anterior. (If you have time, you may want to dissect the eye of the squid, which, though not homologous to ours, shows striking similarity to vertebrate eyes.)

c. Funnel. This is on the ventral side of the body, extending out from the mantle cavity through the collar. It is used in jet propulsion.

2. Internal. With scissors, cup open the mantle laterally, all the way from the edge of the collar to the place where the mantle attaches to the body. Pin back the mantle, and observe:

a. Gills. Paired, lateral, feathery.

b. Gonads, if present, lie partly covered by the gills, attached to the body of the squid.

c. Pen, or shell of the squid, is on the inside of the mantle, long, flat and somewhat brittle. This is the "cuttlefish bone" that is fed to pet birds. Determine the extent of the pen---how far it extends anteriorly-posteriorly, and how far laterally and ventrally.

d. Very little can be learned from internal dissection of the gut, but you can find the whitish rectum that extends a short way into the funnel.

e. The ink sac lies dorsal to the rectum, and is probably black. The squid ejects ink through the funnel when startled.

Discard the squid when you have finished dissecting it.

3. See the demonstrations of Cephalopods in the hall case.

Cephalopods have modified the basic mollusc plan a great deal, to fit a new kind of orientation. This is not so unusual as you might think: humans have done so also, as you will see by mentally comparing your structure with that of a dog. It is helpful in understanding molluscs if you will compare the structural orientation (as in the embryo) with the functional orientation (as in the adult) of a squid:

Structure:	Structural orientation:	Functional orientation:
Pointed end of mantle	dorsal	posterior
Tentacles and arms	ventral	anterior
Head, mouth and eyes	anterior	anterior
Funnel	posterior	ventral

II. ECHINODERMATA.

These are our closest non-chordate living relatives, and as such are of some theoretical interest; but they are entirely marine, have little practical value to us, and therefore our interest here is only theoretical. Members of this phylum form their mesoderm by out-pocketings of the gut, in the same way that primitive Chordates do. All members begin life as bilaterally-symmetrical larvae, but they undergo a "Cataclysmic metamorphosis" and become radially symmetrical as adults. As adults, they show several special Echinoderm characteristics: a water-vascular system, tube-feet, a spiny skeleton embedded in the skin, etc. They are confined to salt water, and must be, because they use sea water as their circulating fluid, and are in osmotic balance with the marine environment.

A. A STARFISH, ASTERIAS SP.

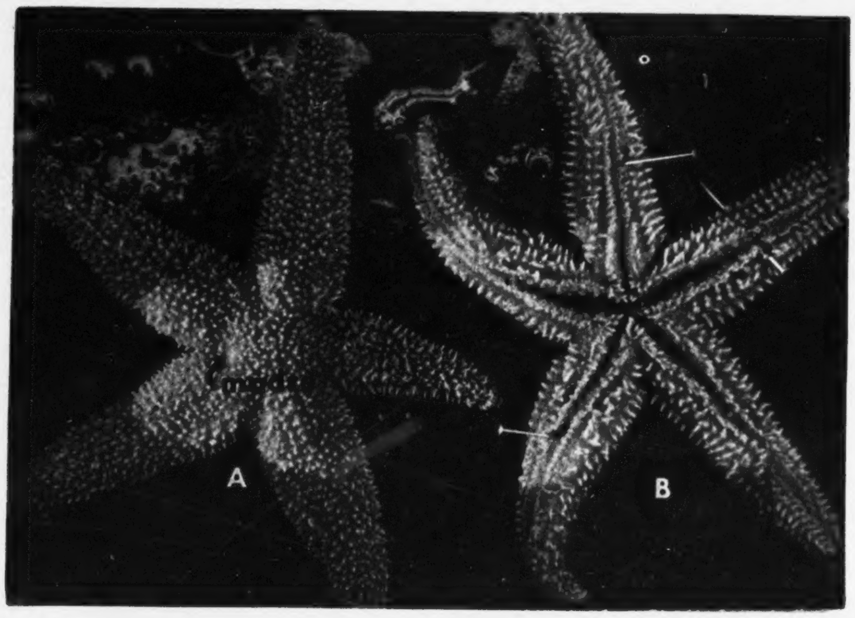


Fig. 9.5 Preserved starfish Asterias x½. A aboral (top) view, B oral (lower) view. madre = madreporite.

Dissect a starfish under water as far as possible. Discard when finished.

1. External. Notice that the radial symmetry is not quite perfect---it is marred by a madreporite, a small flat circular structure in the angle between two of the arms. Sometimes you can find the anus, in the angle between the next-clockwise pair of arms. The madreporite is the external opening of the water-vascular system, through which water is taken in to be used as circulating fluid.

As with all radially-symmetrical animals, ordinary terms of orientation should not be used, but instead use the terms oral and aboral.

Remove a small piece of skin from the animal and examine with the binocular microscope to see the spines of the skin that give the phylum its name (echino- = spine, -derm = skin). If you look closely, you will also see projections of soft tissue between the spines, which act somewhat as gills.

On the oral side find the mouth in the center of the central disc. When the animal eats, it everts its stomach through the mouth, and then the stomach surrounds the food. In spite of the fact that the starfish has an anus, most undigested wastes are expelled through the mouth the next time the stomach is everted.

Notice the groove down the center of each arm. Dozens of small tube-feet fill the groove. With the binocular, find the sucker disc on the end of a tube-foot. These structures, which are connected to the water-vascular system, have the major function of locomotion, by pulling the body along.

2. Internal. With a scalpel, cut one arm into several short sections. Notice that the groove in the arm is made of many ossicles ---hard rods---which meet like the rafters in a roof, and that the tube-feet project from between the ossicles. All the tube-feet connect to a canal that runs just at the peak of the rafters.

The interior of the arm is filled with masses of two kinds of tissue. Distally are the gonads, and proximally are the digestive glands. Particles of food from the stomach enter the digestive glands, and are digested by the cells that line the glands. Does this seem like a primitive or an advanced condition to you?

Dissect away the body around the mouth, and find the stomach, which fills most of the central disc. You may also find the stone canal which carries water from the madreporite, and the ring canal that connects the stone canal with the radial canals in the arms. The radial canals are the ones that run along the peaks of the ossicles in the arms.

B. A SEA URCHIN.

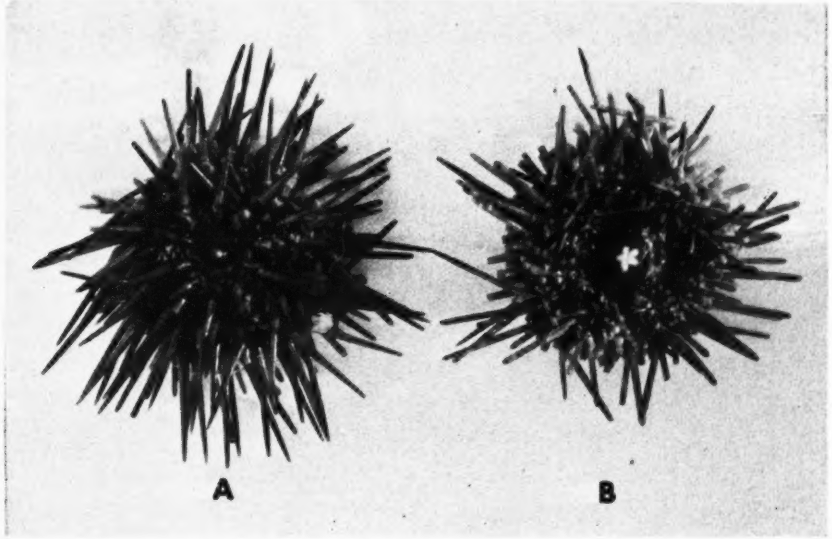


Fig. 9.6
Preserved sea urchin, x½.
A aboral (top) view,
B oral (lower) view.

This animal is like the central disc of a starfish that has lost its arms. Is the animal still divided into fives? Can you find the tube-feet? Is the shell flexible or firm? What does this tell you about the spines of the skin? What do you think is the origin of the long spines that cover the body? This animal browses along shorelines, chewing and swallowing material there. If you have time, dissect the sea urchin, and compare it with the structure of the starfish. You will be especially interested in finding the device it uses to chew food: it has the elegant name of Aristotle's lantern.

C. A SEA CUCUMBER. This animal is like a sea urchin lying on its side. Is the animal still divided into fives? Can you find the tube-feet? Is the shell flexible or firm? What does this tell you about the spines of the skin? This animal lies in mud or sand, eating whatever comes along. You will find little value in dissecting a sea cucumber, because its interior is soft and pulpy.

D. SEE THE DEMONSTRATIONS OF OTHER ECHINODERMATA IN THE HALL CASE.

III. CHORDATA.

One of the most interesting evolutionary stories in biology is that of the development of vertebrate structure from simple, unpromising chordate beginnings. Since all the animals that participated in this development have long since died, we must tell the story only by inference, using examples taken from groups that are still living: the young and adult amphioxus, the lamprey, the frog. This week we take a good look at the amphioxus and some other primitive chordates, and next week we study some vertebrate structure. Next term we will go into the structure of vertebrate animals, including humans, in greater detail.

As you study the chordates, including the vertebrates, keep in mind the three important chordate characteristics:

1. Notochord, which induces the embryonic nerve cord to develop dorsal to it, and then acts as a stiffener for the body.

2. Nerve cord, dorsally-situated and tubular.

3. Pharyngeal gill slits, acting as portholes from the gut to the outside of the body; primitively used in water-straining, later used in respiration.

Also remember that the mesoderm (and only the mesoderm) of the body is segmented, having arisen from pouches of the gut, and that this mesoderm gives rise to segmentally-arranged muscle segments.

A. AN AMPHIOXUS, SUBPHYLUM CEPHALOCHORDATA.

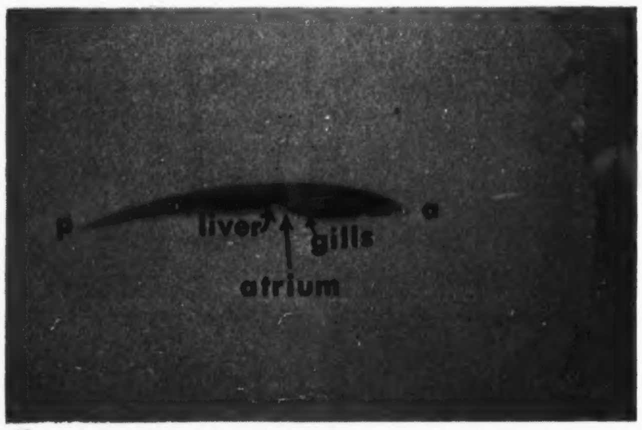


Fig. 9.7 Amphioxus, whole mount, x20. These small, immature animals are on slide #15 (see 2, opposite).

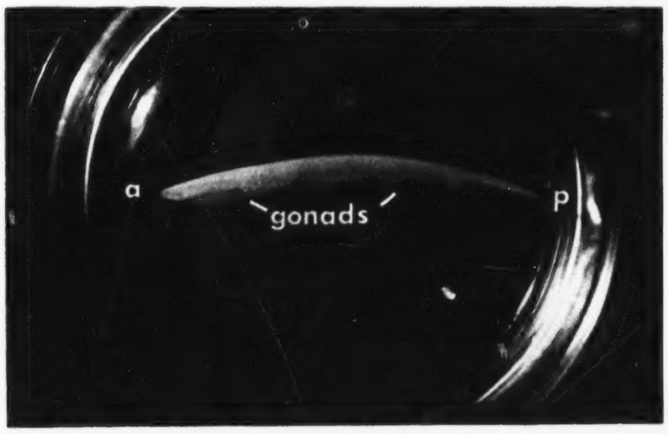


Fig. 9.8 Mature amphioxus, x1. These are 20 times longer than 9.7, and sexually mature (see 1, below).

Experience has shown that the amphioxus is very difficult for students to understand, so follow the directions and photographs carefully, and if you do not see what you should, ask for help.

1. Preserved adult. Put a specimen in a Syracuse watch glass, cover with water, and observe and dissect under a binocular dissecting microscope. For dissection, sharpen your scalpel and needles razor-sharp, or use a single-edge razor blade for cutting.

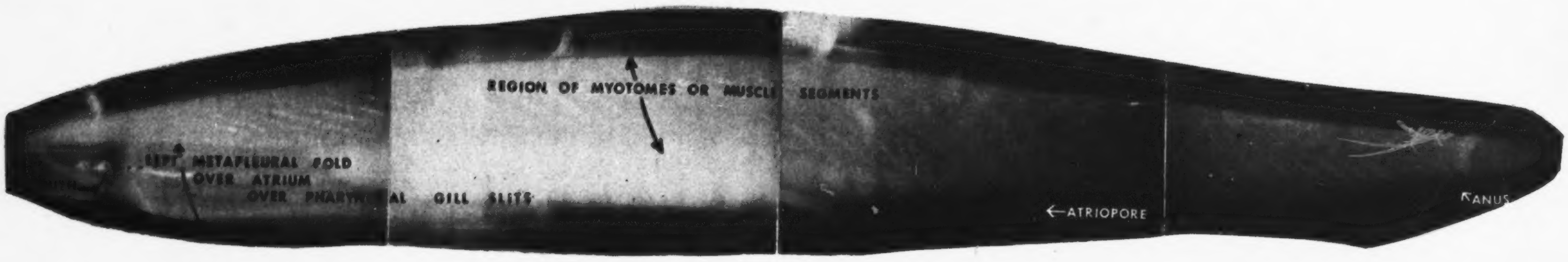

REGION OF MYOTOMES OR MUSCLE SEGMENTS
LEFT METAPLEURAL FOLD
OVER ATRIUM
OVER PHARYNGEAL GILL SLITS
←ATRIOPORE
↖ANUS

Fig. 9.9 Whole amphioxus, x5, as seen from the left side of the animal.

First become oriented, using the labeled photograph as a guide. The most difficult thing for students to comprehend is that the gill slits do not open directly to the outside of the body, because they are covered by some extra folds of the body, called the metapleural folds. These folds enclose the gills in much the same way that the mantle of the squid encloses the body.

Make a mid-ventral, longitudinal incision all the way from the mouth to the atriopore. This is the line along which the metapleural folds originally fused after growing down from the sides of the body. Lay the metapleural folds back. Most of the gonads (testes or ovaries) will remain attached to the folds. Notice that you are not yet truly inside the body of the amphioxus, but have only penetrated into the atrium. Toward the posterior end of the atrium, notice that the pharynx with its gill slits is displaced somewhat to one side of the cavity, and the liver has grown forward from the gut, to lie in the atrium also. Since the liver is still inside the body cavity at this point, what would you expect to find surrounding it? Dissect out the rest of the liver, and also if possible follow the gut posteriorly to the anus. Probe dorsally from the region of the pharynx and try to find the stiff, rod-like notochord that lies just dorsal to it. Do the myotomes (muscle segments) appear to be attached to the notochord?

2. Whole mount, slide #15. The specimens that are mounted are small, sexually-immature amphioxus, that have been cleared (made translucent) and stained so you can see more of the internal structure.

   a. Orient yourself, particularly with regard to the mouth, gill slits, atripore, anus, and liver. There are no gonads, and the myotomes are transparent.

b. Find the rod-shaped notochord, which is probably not stained. The best way to be sure you have the notochord and not something else is to follow it to the very anterior end of the animal: the notochord extends farther anteriorly than any other structure ---sometimes it even sticks out through the skin during the process of making the slides.

c. The nerve tube lies just dorsal to the notochord. You can see it best at the anterior end. The many tiny purple spots are the nuclei of nerve cells, which are large enough to see under low power. Look for photoreceptor cells, much larger, darker, and on the ventral side of the nerve tube. What is their function? How are they distributed along the nerve tube?

d. Dorsal to the nerve tube, find the dorsal fin and its fin rays that support it. Is the fin continuous or interrupted? Can you find where it leaves off and the caudal fin (tail) begins? Or where the caudal fin stops and the ventral fin starts? How far forward on the ventral side does the ventral fin extend? How far forward do its fin rays extend? You will notice either in this slide or in the slide of cross-sections (below), that the anterior part of the ventral fin is not a single, but a double structure. Where does this division occur?

3. Cross sections, slide #16. The slide has several selected cross-sections of amphioxus mounted on it. Using the knowledge and terminology from above, study these cross-sections. Determine from what part of the body each was taken, and what the organs are on each. Some things can be seen on the slide that cannot be seen in the whole animals, such as:

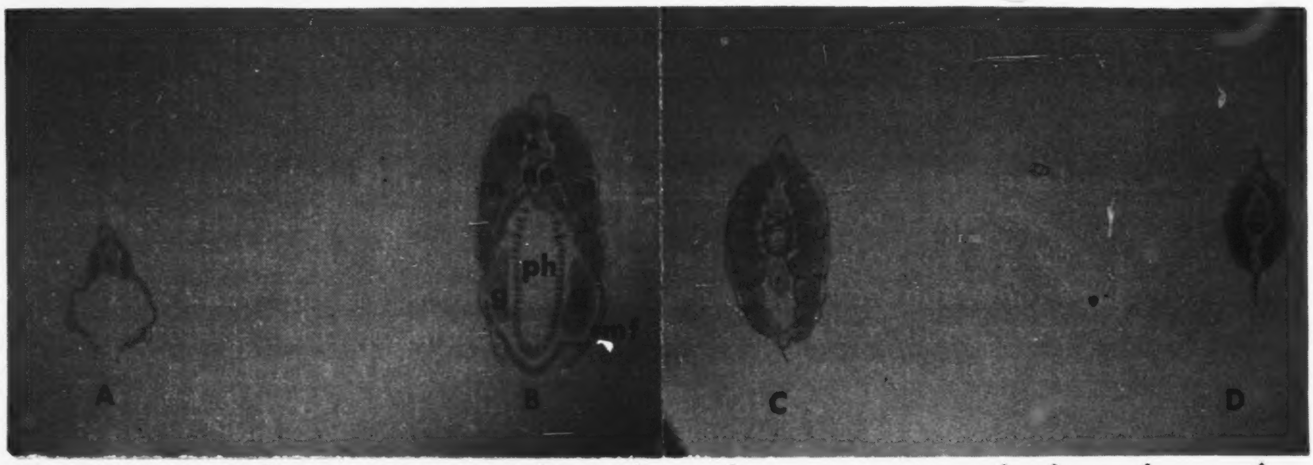


Fig. 9.10 Four sections through amphioxus, x20. A through mouth, B through pharyngeal region, C through intestinal region, D through caudal or tail region. g = gonads, m = myotomes or muscle segments, no = notochord, nt = nerve tube, ph = pharynx.

a. The nerve tube is truly tubular, throughout its length.

b. The cells that surround the gut are tall columnar cells, perpendicular to the lumen of the gut.

c. The gill slits are actual openings between the lumen of the pharynx and the atrium.

LABORATORY WEEK 10:
LANDMARKS OF VERTEBRATE STRUCTURE

This continues the work begun last week in chordate evolution. Keep in mind the three important chordate characteristics, and see what happens to them in more complex vertebrates.

1. Notochord.
2. Nerve tube.
3. Pharyngeal gill slits.

In the vertebrates, the above characteristics have evolved further:

1. The skeleton, including the vertebrae, arose from some genetic change that allowed any mesodermal derivative to produce bone; and particularly it is found in the dermis of the skin, between the myotomes, and around the notochord.
2. The brain arose from an anterior swelling of the nerve tube.
3. Gills, and later lungs, arose from the pharyngeal gill slits and pouches.
4. Separate fins arose from the fin-folds such as you found in amphioxus, and later limbs arose from the fins, fin rays and muscles.

Notice that all individuals studied this week belong to the phylum Chordata, subphylum Vertebrata.

I. LARVA OF A LAMPREY.

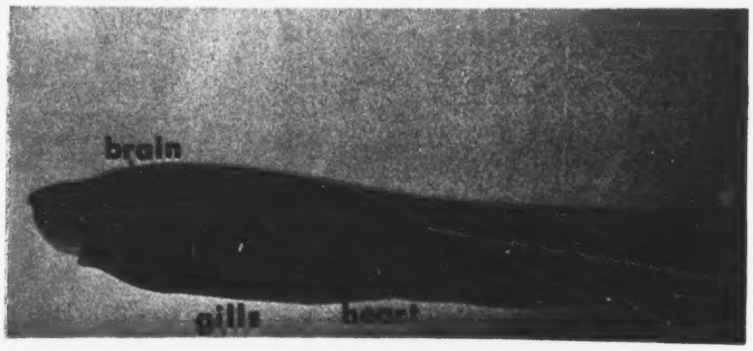


Fig. 10.1 Ammocoetes larva of lamprey, x20.

This is used to show the transition between primitive chordates and the vertebrates. Slide #17 is a whole mount of a lamprey larva, called "Ammocoetes larva". Examine it with binocular microscope and low power of a compound microscope. Do not use high power on this slide!

These seem so similar to amphioxus that you will think at first that that is what you are looking at. Discover the following similarities and differences, and any others that you can find:

Body Part:	Similarities	Lamprey larva differs in this respect:
1. Pharynx:	gill slits present.	Gills fewer in number, no atrium or folds.
2. Gut:	liver present, gut straight.	More divisions in the gut.
3. Anus:	opens anterior to tail.	More tail posterior to anus.
4. Fins:	present.	Somewhat different arrangement.
5. Myotomes:	present, cells longitudinal.	Segments more "W" than "V" shaped.
6. Nerve tube:	present.	Anterior end swollen into brain.
7. Notochord:	present.	Not present in "head" region.

Some of the slides of lamprey larvae will show a swollen area just anterior to the liver, which is the heart. Although these slides do not have the blood vessels injected, in places you can detect the ventral aorta carrying blood forward from the heart, then dividing into branchial arteries carrying blood dorsally through a gill, then these fusing into the dorsal aortas that carry blood posteriorly. (You will see this better in the injected adults, later.) How does this route of circulation compare with that of the Annelida and Arthropoda?

II. ADULT LAMPREY.

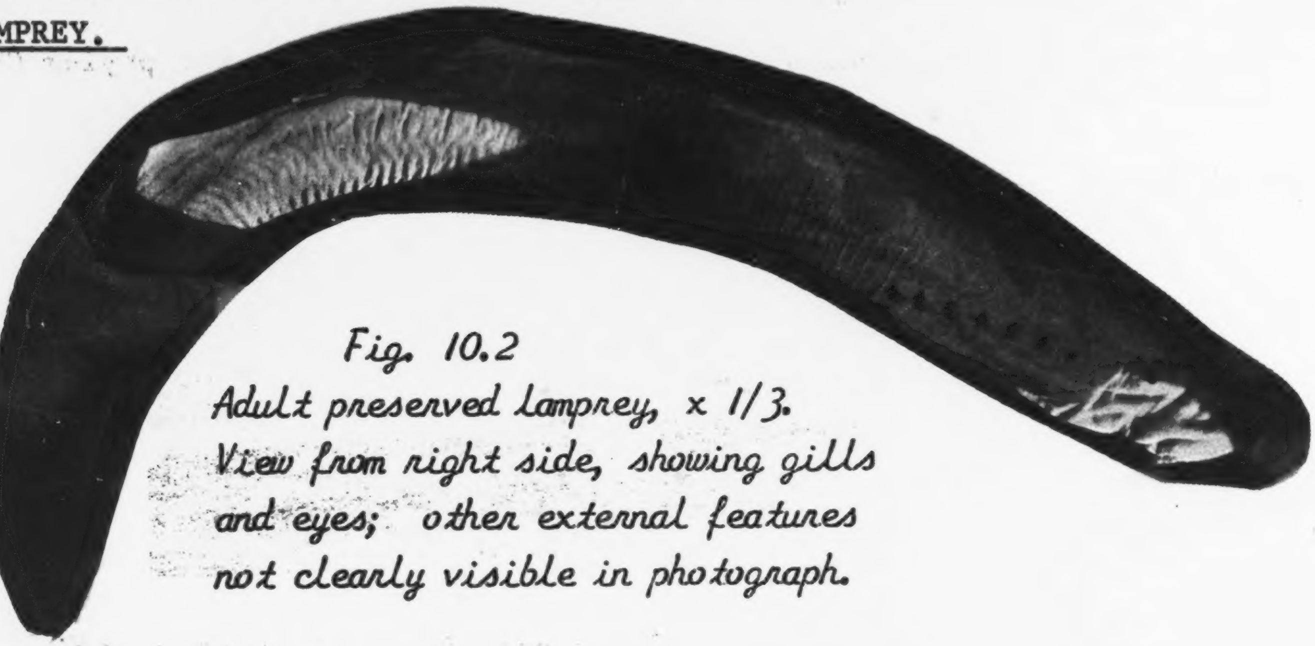


Fig. 10.2
Adult preserved lamprey, x 1/3. View from right side, showing gills and eyes; other external features not clearly visible in photograph.

Although we study the lamprey in order to see "primitive vertebrate" characteristics, you should understand clearly that it is not much more primitive than you are in most of its structures. We humans do not have any lampreys in our family tree; but we and the lampreys have common ancestors, and in some ways the lampreys have maintained a few of the characteristics of the common ancestors with negligible changes. It is those characteristics that we will examine, primarily: lack of jaws; lack of limbs; old-fashioned circulatory system; maintenance of part of a notochord; tiny vesicular brain; myotomes.

A. EXTERNAL MORPHOLOGY.

Examine your preserved specimen, noting the following:

1. A round mouth; teeth; but no jaws at all. It is the round mouth that gives the general name "cyclostomes" to this group: cyclo- = round, -stome = mouth.

2. A cylindrical body, without scales or bony plates. Just skin. The body is vaguely divisible into head, trunk and tail. Notice the arrangement of the fins.

3. Gill slits (how many?) open directly to the outside, without metapleural folds.

4. Sensory organs:

    a. Two eyes, without lids. (Under the microscope, you would see the retinas of these eyes lined with photoreceptor cells like those of amphioxus.)

    b. One pineal body, the rudiment of a median eye, lying mid-dorsally under a layer of thin transparent skin.

    c. One median olfactory pit, used in tasting and/or smelling. This does not connect with the mouth in any way.

    d. Two lateral lines, one on each side of the body. These are really rows of sensory pits, used probably in detecting changes in water pressure.

5. An anus, located ventrally, anterior to the tail.

6. A urogenital sinus, posterior to the anus, through urinary wastes and products of the gonads pass.

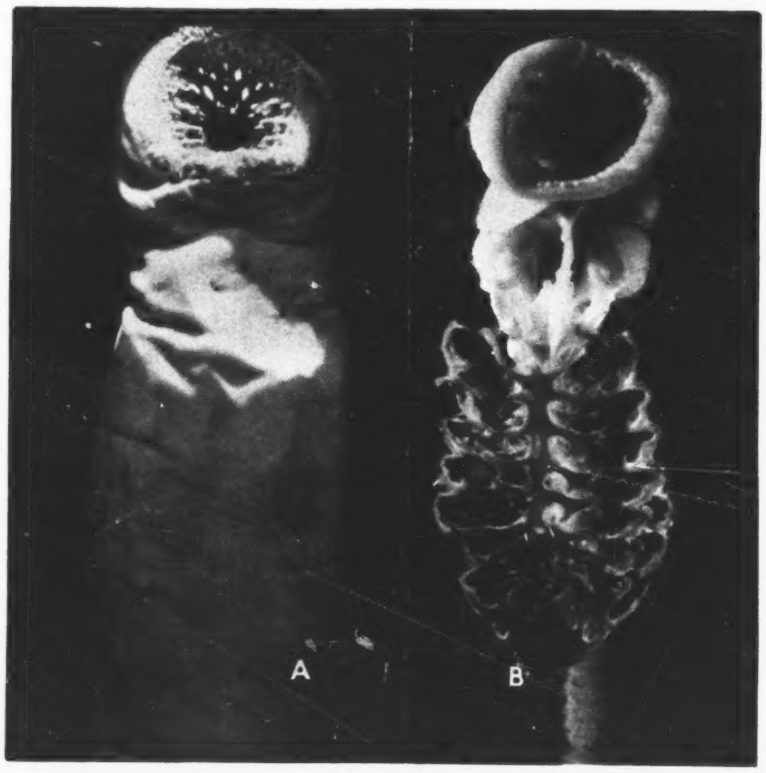


Fig. 10.3
Composite showing whole lamprey and lamprey skeleton, ventral views. Gill slits open between arches of "branchial basket". Lamprey skeleton is entirely made of cartilage. See also #4, page 10-5.

B. INTERNAL MORPHOLOGY. When you have completed your dissection this week, do not dispose of the specimen, but put a label on it and save it for next term, by putting it into the jar of formalin provided. Next term we will dissect several parts of the lamprey that we are not going to do now.

1. Myotomes. Dissect away the skin from one side of the body about halfway back, and then carefully dissect into the muscle until you have determined the arrangement (three-dimensional) of one myotome. You will find myosepta between the myotomes. The process of exposing one single myotome is not as easy as it sounds, so work carefully. Determine, also, the direction that the muscle fibers run within the myotome. When a muscle fiber contracts, what action does it have? Dissect all the way to the notochord, and examine it carefully, particularly for muscular attachments.

2. Gills. Dissect the gills on one side of the body, in such a way that you can follow the route of water from the mouth, through the pharynx, and through the gills. This area will not be what you expect, at least from your examination of the amphioxus and lamprey larva. Respiration has been almost entirely sealed off from digestion: gills do not open from the pharynx proper, but from an extension of the pharynx. Lampreys are not water-strainers, but predators. When lampreys feed, they remain attached to their fish host for long periods of time, and the mouth is pressed firmly to the host. How do you suppose water circulates over the gills while the lamprey is feeding?

Fig. 10.4
Lamprey, opened with a median sagittal cut. This will give you the location of some of the important landmarks, to help with your dissection.

3. Circulation. Dissect just posterior to the most posterior pair of gills, and find the heart lying in its pericardial sac. The heart is two-chambered, and twisted into a kind of S-curve, so that the ventricle lies to the right and the atrium lies to the left. You should find a thin-walled sinus venosus attached to the atrium and a thick-walled conus arteriosus attached to the ventricle. Blood flows from all parts of the body into the sinus venosus, then into the atrium, then is pumped by the ventricle through the conus arteriosus out to the gills.

a. The ventral aorta is an anterior continuation of the conus arteriosus. It carries blood anteriorly, between the bases of the gills, and gives off branches into the septum between adjacent gills.

b. The afferent branchial arteries then carry the blood to capillaries in the gills.

c. The efferent branchial arteries pick the blood up from the capillaries and carry it dorsally, where all these efferent branchials fuse.

d. The dorsal aorta is the result of the fusion of the efferent branchials. It lies just ventral to the notochord, carrying blood posteriorly and giving off branches to all parts and organs. Note: the branches are paired if they are lateral and go to somatic organs like muscle, urinary and reproductive organs; and are single if they go to visceral organs such as the digestive system.

e. Slightly laterally, on each side of the dorsal aorta, find the cardinal veins, which pick up blood from the somatic organs served by the paired arteries. Follow the cardinal veins forward to the heart region, where they turn medially and become the ducts of Cuvier. These collect blood from several other places, and then carry it to the sinus venosus.

f. Blood in the visceral organs (served by the unpaired arteries) is picked up by a single large vein, the hepatic portal vein, and carried anteriorly. The liver interrupts the passage of this vein, and the blood vessel is broken up into capillaries in that organ; then the capillaries re-form into the hepatic vein which carries blood to the sinus venosus via the duct of Cuvier.

4. Skeleton. Lampreys have lost the ability to produce bone, so you will find only cartilage in its skeleton, and this is too difficult to dissect to bother with.

a. Examine the demonstration of the "branchial basket" of a lamprey. This is the cartilagenous remnants of the bony supports of the gills.

b. Dissect out enough of the notochord to see that it appears to be cartilage in makeup. Do you find anything like vertebrae, ribs, etc.? Make a longitudinal section through a piece of the notochord, to see its structure.

Aside from these two observations, nothing further will be done with the lamprey's skeleton. Instead, we will study the skeleton of a frog, that can still produce bone.

III. LARVA OF A BULLFROG.

This is used to show the transition between primitive aquatic vertebrates and more advanced terrestrial vertebrates with appendages.

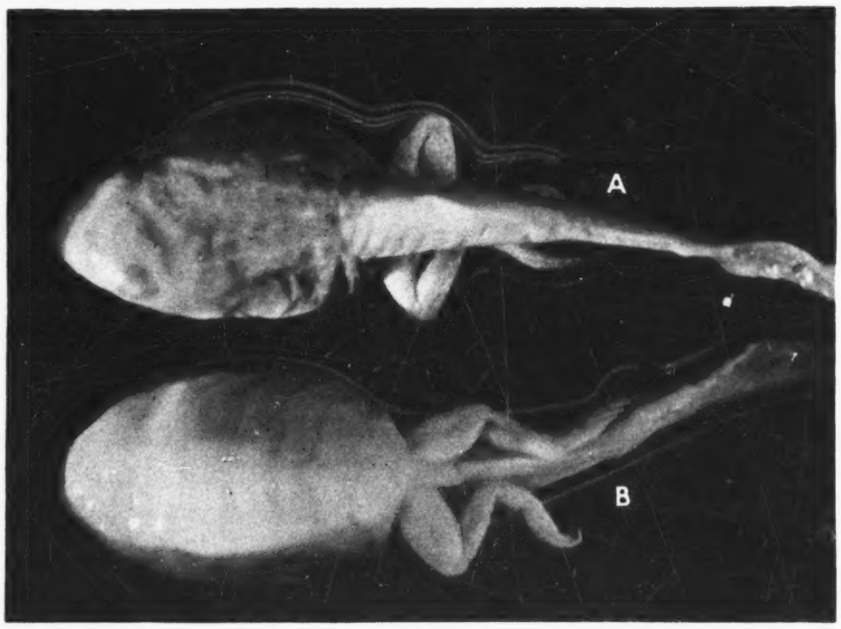


Fig. 10.5
Bullfrog larvae.
A dorsal view,
B ventral view.

A. EXTERNAL MORPHOLOGY. Examine a preserved specimen, noting the following:

1. A mouth, with jaws.

2. The body. Compare its shape, divisions and fins with that of the lamprey. Do you find scales, bony plates, etc.?

3. Gills. As in the amphioxus, the gills are covered with external folds---in the frog larva these are called an operculum. Is there anything that compares with the atripore? Where? This is called a spiracle. Dissect away the operculum, and count the gill slits.

4. Sensory organs. You should be able to find two nostrils, two eyes, two lateral lines, and possibly one pineal body. Compare with the lamprey.

5. Anus. Compare its location with that of the lamprey.

6. Limbs. How many? Where are they located? What relation do they have to the fins? See if you can determine where their musculature is attached.

B. INTERNAL MORPHOLOGY. Open your specimen with a midventral cut, and also by examination of the myotomes.

1. Uncoil and measure the gut, and compare it with the length of the body. Save this information until next term, when you do the same with an adult frog.

2. Where is the heart located, with reference to the gills? Compare this with the lamprey.

3. The posterior set of gill pouches does not break through to the outside of the body, but remains as pouches in the larva. At matamorphosis, these will grow posteriorly to become lungs. You can probably find these pouches, with careful dissection.

4. Notice that there are more divisions to the digestive system than in the lamprey: esophagus, stomach, small intestine, large intestine.

5. Cut transversely through the tail of the larva, and examine the cut surface. You should be able to distinguish the notochord with a vertebra developing around it; the nerve tube dorsal to the notochord; and the dorsal aorta just ventral to the notochord.

IV. SKELETON OF A BULLFROG.

Ancient vertebrates, ancestors of both lampreys and frogs, possessed the ability to make bone---old ostracoderms were both covered and filled with a great assemblage of bone. The tendency in vertebrates seems to be, however, to decrease that ability unless it is specifically needed. Aquatic forms need little bone. Lampreys and tadpoles have none or little. Terrestrial forms such as the frog need bone as support, so now we come to an animal that has enough skeleton to study.

On each table you wind several cleaned and articulated frog skeletons, and also some loose human vertebrae. Study and record.

A. THE MAJOR DIVISIONS OF THE FROG SKELETON.

1. Axial skeleton: bones of the skull and the vertebral column.

2. Hyoid apparatus: what is left, in the adult frog, of the fish-type gill apparatus. (See the "branchial basket" of the lamprey.) The frog's hyoid apparatus is made up almost entirely of cartilage, and may either be missing entirely in your specimen, or be attached to the end of the mounting box. (See Fig. 10.7)

3. Appendicular skeleton: primarily the bones of the appendages, including the bony girdles in the shoulder and hip region. This also includes the sternum (breast bones).

B. VERTEBRAL COLUMN. The first vertebra is the atlas: it has no transverse processes (see below, #4, for meaning of this term). The atlas has a pair of depressions on its anterior surface, and the condyles of the skull fit into these depressions. The ninth vertebra is the sacral vertebra, and it has large transverse processes onto which the hip girdle is attached. The urostyle is a long bone attached to the posterior end of the sacral vertebra; it is a fused group of larval tail vertebrae.

On a single dis-articulated human vertebra find the following parts:

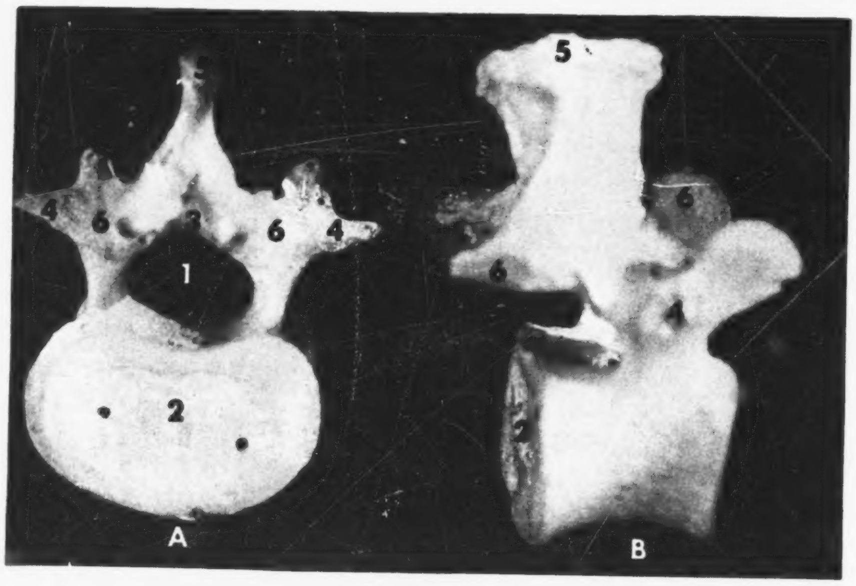


Fig. 10.6
Disarticulated human vertebrae.
A as seen from below (posterior),
B as seen from the right side.

1. Neural canal, the cavity in the vertebra, continuous from one vertebra to another, through which the spinal cord runs.

2. Centrum, the body of the vertebra, which has taken the place of the notochord. The centrum forms the ventral side of the neural canal.

3. Neural arch, the bony U-shaped part, forming the lateral and dorsal sides of the neural canal.

4. Transverse processes, a pair of lateral projections from the neural arch. (Note: a "process" is any projecting part of a bone.)

5. Neural spine, the median dorsal process.

6. Zygopophyses, or articulating processes. There is an anterior pair and a posterior pair. Those of one vertebra rest upon those of the next, to form a joint or articulation, by which the vertebrae are locked together to form a continuous column.

C. SKULL. A general term, used to describe all the bony parts anterior to the vertebral column. The term includes more than just the brain case. As you work, compare the skull with the preserved frog you used the first week, to see where the organs are that are referred to.

1. Cranium: brain case, a small elongated box-like structure which forms the median dorsal portion of the skull.

2. Orbits: the spaces into which the eyes are fastened.

3. Olfactory capsules: anterior to the cranium, between it and the upper jaw. They contain the organs of smell. The external nares open into these capsules.

4. Tympanic rings: support the tympanic membranes (ear drums). These may have been removed in preparing the skeleton.

5. Auditory capsules: extend laterally between the posterior part of the cranium and the tympanic rings; protect and support the organs of hearing.

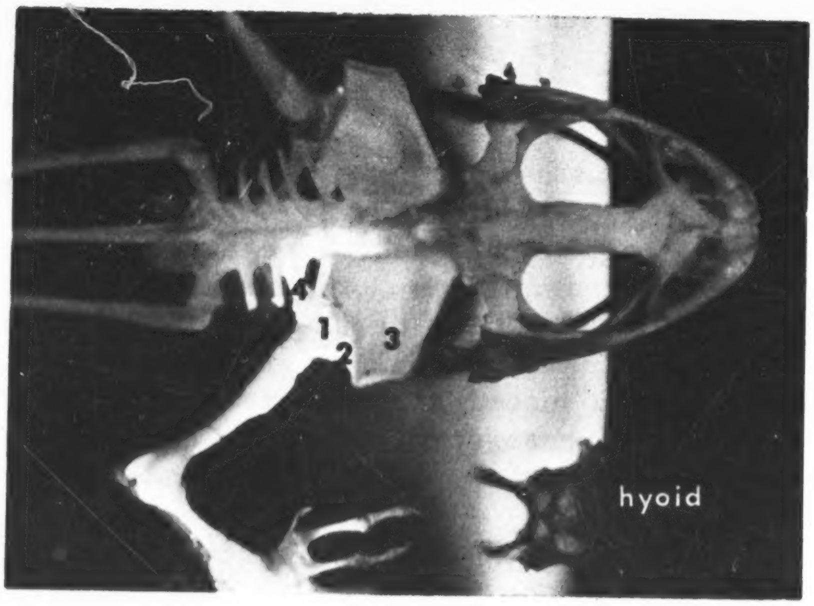
6. The upper jaw is called the maxilla. How is it attached to the rest of the skull?

7. The lower jaw is called the mandible. How is it attached to the rest of the skull?

8. The skull articulates with the atlas by means of two occipital condyles, one on each side of the foramen magnum, the large opening through which the spinal cord passes in connecting with the brain.

D. PECTORAL (SHOULDER) GIRDLE. A group of bones that supports the anterior limbs. It is made up of right and left halves, that meet mid-ventrally where they enter into a close articulation with the sternum. The pectoral girdle plus the sternum make an incomplete ring, nearly encircling the chest, but they do not articulate with the vertebral column at any point.

Fig. 10.7
Frog skeleton, showing part of pectoral girdle. Clavicle does not show in this picture.



1. As a landmark, locate the glenoid cavity, which is a depression into which the bone of the upper arm fits, forming a ball-and-socket joint.

2. The scapula or shoulder blade projects dorsally from the glenoid cavity.

3. The suprascapula is attached to the dorsal end of the scapula. It is very broad and thin.

4. The coracoid projects ventrally from the glenoid cavity. It passes to the mid-line on the ventral side of the body, where it widens and fuses with the coracoid from the other side of the body.

5. The clavicle runs parallel to the coracoid, anterior to it. It is more slender than the coracoid. It is not involved in the glenoid cavity.

E. STERNUM. Find the fused ends of the coracoids and clavicles. Anterior to the region of fusion there are two fused bones: the anterior one is the episternum and the posterior one is the omosternum. Posterior to the coracoids are two more: the anterior one is the mesosternum and the posterior one is the xiphisternum. All four of these, taken together, make up the sternum. In the human, certain ribs have connections to the sternum. Is this true in the frog?

F. PELVIC (HIP) GIRDLE.

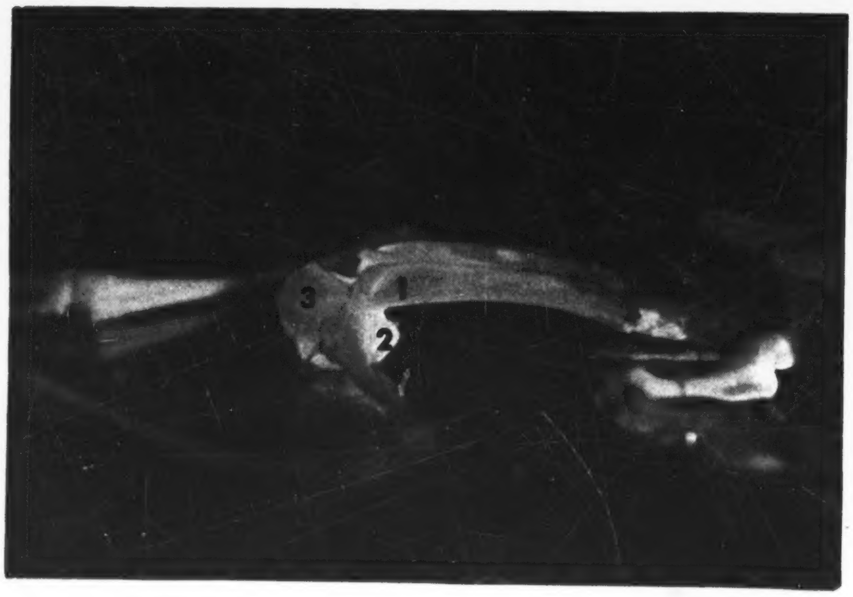


Fig. 10.8
Frog skeleton, pelvic region from right side. Femur fits into acetabulum. One innominate bone is shown, with its three fused bones labelled as below.

The hip region appears to contain one large U-shaped bone, the pelvis, but actually it is made up of six smaller bones, all fused together. Imagine a line down the center of the pelvis, dividing it into equal right and left sides. For convenience we will refer to each half by the term innominate ("nameless") bone. Each innominate bone is in turn made up of three fused bones. Find the acetabulum, the socket used for articulation with the leg. The three fused bones that form the innominate meet in the middle of the acetabulum:

1. Ilium: the long, anteriorly-directed bone that runs parallel to the urostyle, and which articulates with a transverse process of the sacral vertebra.

2. Pubis: small, wedge-shaped, around the ventral border of the acetabulum.

3. Ischium: larger than the pubis, projecting posterior to the acetabulum.

G. BONES OF THE LEGS. Anterior and posterior legs are similar:

Bones of the anterior leg:	Bones of the posterior leg:
1. Humerus, upper arm, articulates at glenoid. Ball-and-socket.	1. Femur, upper leg, articulates at acetabulum. Ball-and-socket.
2. Radio-ulna, fused radius and ulna, in fore-arm. Hinge.	2. Tibio-fibula, fused tibia and fibula, in shank. Hinge.
3. Carpals, several, small, in wrist.	3. Tarsals, two long plus several small, in ankle.
4. Metacarpals, slender, palm of hand.	4. Metatarsals, arch of foot.
5. Phalanges, in fingers.	5. Phalanges, in toes.

V. SKELETON OF A HUMAN.

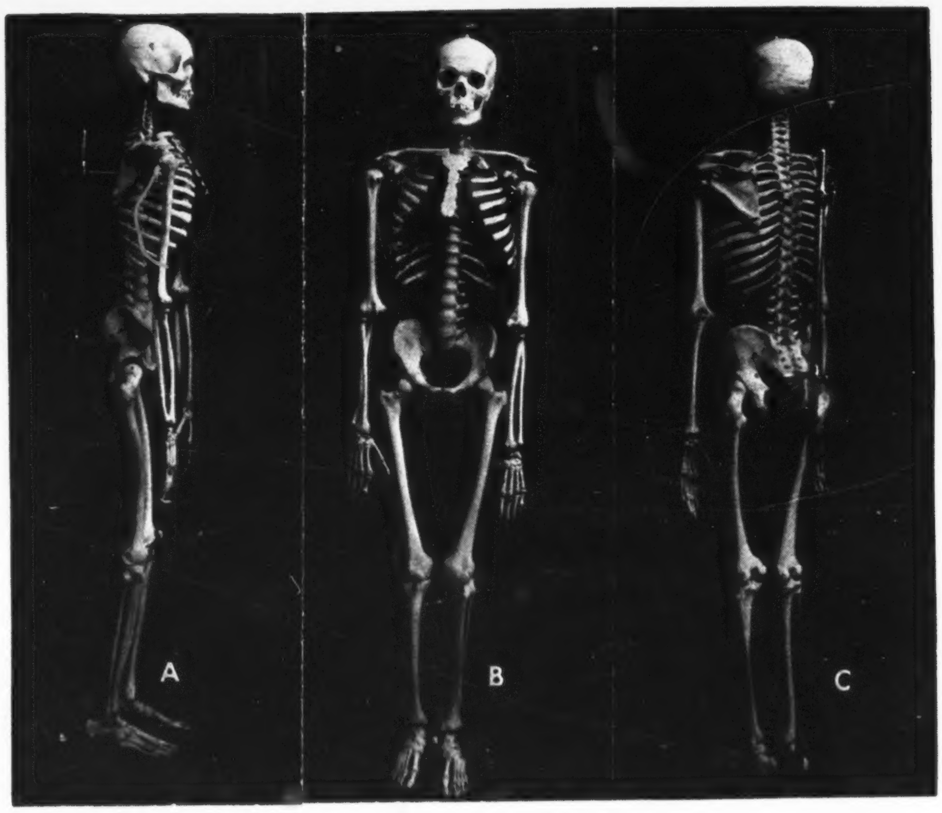


Fig. 10.9
Three views of a human skeleton. Notice that, because of the different posture, terms of body orientation are different also. A is a lateral view, B is anterior (not ventral as in the frog), C is posterior (not dorsal). Terms for upper and lower are superior and inferior.

The laboratory skeleton of a human has had labels attached to it, showing the major bones. There are many similarities between the skeletons of the frog and human, and a few notable differences. Discover as many differences as you can between the two. This is easiest if you take up the regions of the skeleton one by one: vertebral column, skull, pectoral girdle, sternum, pelvic girdle, limbs. Record in your data book.

VI. MUSCULATURE OF A FROG.

Use the frog that you dissected the first week.

A. MUSCULATURE OF THE BACK. Recall the musculature of the lamprey: rows of W-shaped myotomes, muscle fibers arranged antero-posteriorly within the myotomes, the work of the muscles exerted in making lateral motions with the tail. Remove the skin from the back and sides of your frog, and see if you can discover any remnants of this primitive condition. Can you discover myotomes? What direction do the muscle fibers run? Dissect away layers of muscle from the back and sides, to see if your observations hold true in all layers. What do you think is the major work of the back muscles? Of the belly muscles?

B. MUSCLES OF THE LIMBS. When you eat a fish, what kind of musculature do you eat? What kind do you eat in cattle? It is the large limb musculature that makes this difference---people eat frog legs, but would have a rather slender meal dining on the same parts of a lamprey. The limb musculature develops in the embryo from small down-growing slips from myotomes. In a fish such as the perch it is enough to have dorsal and ventral antagonistic muscles for the fins, but in land animals this antagonistic situation becomes more complex. Muscle fibers become gathered into groups called muscles, and each muscle comes to have a distinct action upon the limb; primarily in changing the angle between the bones that make up a joint. Remember: muscles work only by contracting, not by extending; therefore it is necessary for every muscle to have an antagonist. This arrangement is best seen in the shank of the frog.

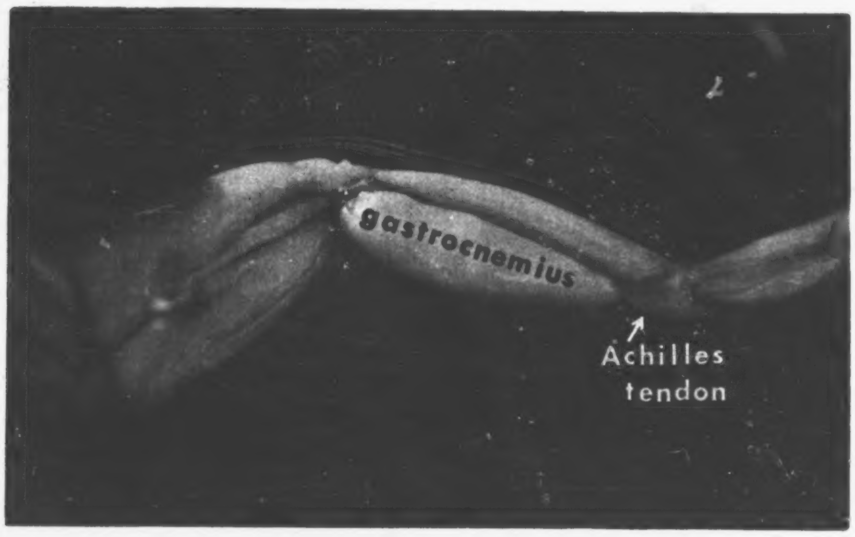


Fig. 10.10
Dorsal aspect of skinned frog leg, x1. This locates the gastrocnemius muscle for you.

1. Parts of a large typical muscle. Remove the skin from one hind leg, and find the gastrocnemius muscle, lying dorsal to the tibio-fibula.

a. Connective tissue covers the muscle with fascia (thin, transparent), connects the ends of the muscle to bones through tendons, and ties the whole muscle together into a structure that is not easily separated.

b. The origin of a muscle is the tendon on its proximal end; in this case, the tendon that attaches the gastrocnemius to the femur.

c. The insertion of a muscle is the tendon on its distal end; in this case, the tendon that attaches the gastrocnemius to the ankle and the foot. This particular insertion is called the Achilles tendon.

d. The middle, swollen part of a muscle is usually called its belly.

2. Connections and actions. Carefully dissect the gastrocnemius muscle, making notes of every connection it has with bones, either through fascia or through tendons. Assuming that the muscle works only in contraction, what actions are brought about by the gastrocnemius? To describe these actions, you will need this terminology:

a. To flex: make the angle at a joint smaller.

b. To extend: make the angle at a joint greater. Antagonistic to flexion.

c. To adduct: bring a limb or part of a limb closer to the midline of the body.

d. To abduct: take a limb or part of a limb farther from the midline of the body. Antagonistic to adduction.

3. Antagonists of the gastrocnemius. Now that you know the actions of this muscle, you should be able to locate other muscles with opposite actions. They need not be located in the shank. Find these muscles, and be able to locate them again. You need not know their names. About how many muscles are required to antagonize all the functions of the gastrocnemius?

OPEN LABORATORY 2

Second Edition
revised

A NEW APPROACH
TO COLLEGE GENERAL BIOLOGY
LABORATORY STUDIES

Second Term of a Two-Term Course of Study

William L. Downing, Ph.D.
Professor of Biology
Hamline University

Published by the author at Hamline University, St. Paul, Minnesota 55101

This second edition is the result of the interaction of the experimental first edition, with the students and Biology faculty at Hamline. A great deal of material has been eliminated and much added, as results of their helpful constructive criticism. In continuing our policy of using as much living material as possible in the laboratory, methods have been devised to use living fruit flies as examples of genetic crosses, and living members of various metaphyte groups are now grown by students in the laboratory.

OPEN LABORATORY 2

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Introduction

I. Equipment Required

All the items listed below are required for Biology 12 General Biology, and are available from the Hamline University Bookstore. All were required for Biology 11, and therefore item #1 is the only new one.

1. A copy of this book, Open Laboratory 2.
2. A copy of the text, Weisz, Paul B., The Science of Biology, edition 3, 1963, McGraw Hill Book Company, New York.
3. A bound, cross-lined laboratory data book.
4. A dissecting kit of good quality.
5. A packet of lens paper.
6. About a dozen microscope slides and cover glasses.

It is recommended that you have the following:

7. Plain white paper for laboratory reports.
8. A copy of the book Brenner, Robert M., Study Guide for Weisz: The Science of Biology, edition 3, 1963.

II. The Course

Biology 12 is a continuation of the course begun with 11. The philosophy behind the course remains the same, and the methods of carrying out that philosophy remain much the same as well. The lectures, text assignments, laboratory practicals and lecture quizzes continue as before; the Open Laboratory system and the method of checking into and out of the laboratory continue unchanged.

Some differences: By the nature of the subjects presented and the materials studied, there will be less work with living forms and more dissection; less experimentation and more examination. There will be greater emphasis placed on good laboratory records and less emphasis placed upon writing reports. Your laboratory data books will be checked by your conference instructor on a regular schedule, approximately every two weeks.

III. An Option: Independent Project

After consultation with your conference instructor, and with his prior approval, you may omit any one, two or three weeks' laboratory work, and instead undertake an independent project.

This may be concerned with any materials previously used in the course, or other materials you know we have.

The following examples may be helpful in your thinking:

a. You may wish you could have continued your study of slime molds, or of planaria regeneration, beyond the time limits we had last term. Your instructor may approve a plan for their more extensive study.
b. You might want to go more deeply into chemical composition of foodstuffs, or of living material, or in vital staining, than you did last term.
c. After studying Weisz Chapter 24 ("Reproduction: Monera and Protista"), you may want to carry out reproductive studies on some form used last term.

How to proceed with your independent project, if you elect to do one:

a. Study the laboratory directions in this syllabus, to see which one or ones you could omit without harming your biological education.
b. Prepare a written statement for your conference instructor, including:
    (1) Which laboratory week(s) you plan to omit, and why you feel you can do so.
    (2) A plan for your experimentation, including at least the following ---

        Hypothesis or question you plan to check.
        What materials you will require.
        What methods of study you plan to follow.
        In what kind of terms you plan to state your results.

c. Obtain your conference instructor's approval before beginning experimentation.
d. Carry out the research at any time you wish.
e. Do not take the laboratory practicals for the week(s) you plan to omit. The grade assigned to your Report (see below) will take the place of the grade(s) on the practical(s). The data in your data book will take the place of the data from the week(s) you omit.
f. Write up a Report of your experimentation, using the proper form, and hand it in to yourconference instructor no later than one week before the end of the term.

This plan of an optional independent research project is designed to accomplish two desirable ends: to avoid repetition of work previously done by students in high school and to get students doing independent research under supervision, early in their biological careers.

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LABORATORY WEEK 11:
ARTERIES OF A BULLFROG

The dissection specimen is a preserved, injected giant bullfrog, Rana catesbeiana. Take a specimen from the barrel, and keep it in a closed plastic bag in your locker except when you are dissecting it. Every person should do his own dissecting. Use dissecting pans while working, but do not put the dissecting pan in your locker.

The bullfrogs have had their blood vessels injected with colored latex, arteries red and veins blue. Capillaries are not injected. Note this: because of technical difficulties in injecting, many of them will have no latex in the large veins around the heart, so those will appear much smaller than other vessels, and will have a gray-ish color. This is important to watch, because in your preliminary dissection you are liable to spoil these veins without noticing them.

I. PRELIMINARY DISSECTION

It will be necessary to open the body cavity in order to examine the contents. Repeat the procedure of opening the body cavity that you used with Rana pipiens (see Laboratory Week 1, VI-B), with this exception: when you cut through the muscle covering the abdomen, cut slightly to one side of the midline (see Fig. 11.1). The abdominal vein is attached to the dorsal side of that muscle, and cutting up the midline would spoil it. After you have the abdominal cavity open, separate the abdominal vein (colored blue) from the muscle and leave it in place across the intestine when you turn the muscle flap back (see Fig. 11.2).

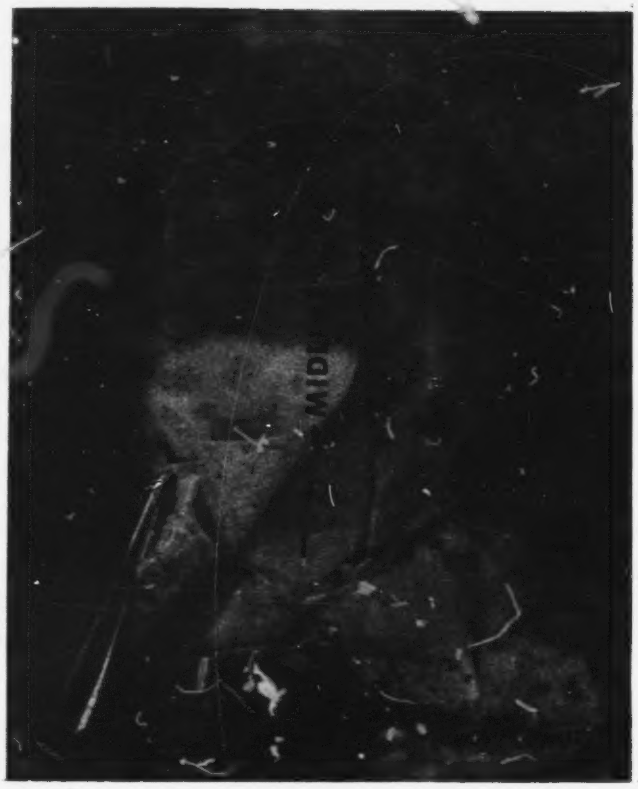


Fig. 11.1 Opening the skin.

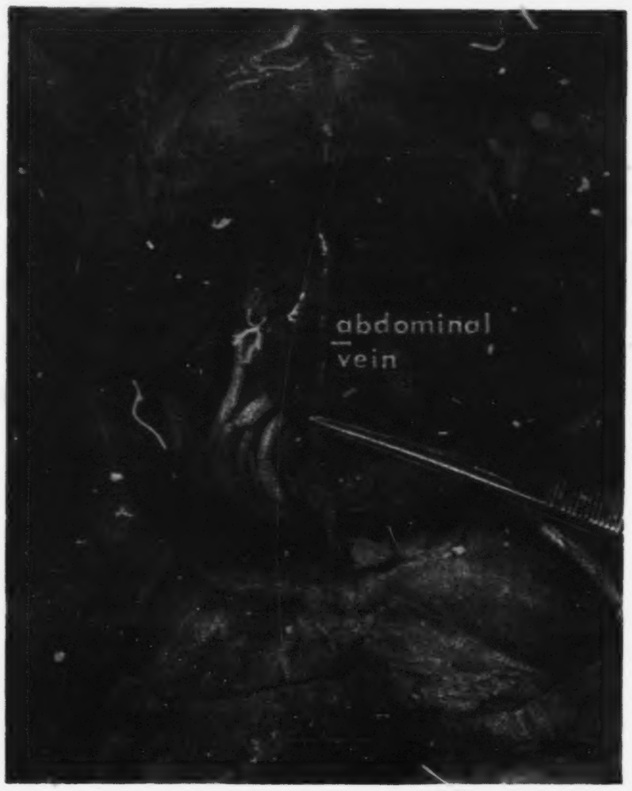


Fig. 11.2 Leaving the abdominal vein in place.

Cut through the pectoral girdle, using heavy bone-clippers if necessary (see Fig. 11.3), but do not pull the two sides of it away from each other. If you do, you will break several large veins attached to the heart. Instead, after you have laid the viscera bare, remove, bit-by-bit, the entire pectoral girdle. (see Fig. 11.4) Watch the blood vessels as you do so, especially the gray-ish veins mentioned above.

II. CIRCULATORY SYSTEMS

Any circulatory system moves fluid around a body. In highly-organized animals, the fluid is usually some kind of blood; and in animals with a large oxygen requirement, the blood ordinarily has some kind of oxygen-storing pigment such as hemoglobin. Although insects survive well with an "open" circulatory system, vertebrates' systems are almost entirely "closed". This means that the blood never escapes from the series of closed tubes into the general tissues of the body. Nevertheless, a circulatory system is in intimate enough contact with the cells of the body to provide them with nutrients, moisture and oxygen, and to carry away their metabolic wastes. Without proper circulation, body cells of metazoa would die quickly.

There are four kinds of blood vessels in vertebrates:

1. A heart, the major organ used in pumping blood.

2. Arteries, thick-walled and muscular, carry blood from the heart.

3. Capillaries, microscopic-sized vessels with walls only one cell thick, through which nutrients and gases and wastes pass readily, but which still keep the blood contained. Arteries carry blood to capillaries.

4. Veins, thinner-walled than arteries; carry blood from capillaries back toward the heart. There are two kinds,

    a) If they carry blood directly to the heart, without any intervening capillary beds, they are ordinary veins.
    b) If they carry blood from one set of capillaries to another set of capillaries, they are called portal veins. In the frog there are two portal veins, studied in Lab. Week 12.

Structurally, all these vessels are essentially similar, and merge gradually into each other.

At the end of two laboratory weeks, you should be ready to take an individual oral examination over the arterial and venous systems of your own bullfrog specimen. You should be able to identify all veins and arteries mentioned in Lab Weeks 11 and 12, and should also be able to trace blood from one place in the body to another. Oral practical examinations will be given by appointment during your conference or at some other time set by your conference instructor. See your instructor for the appointment.

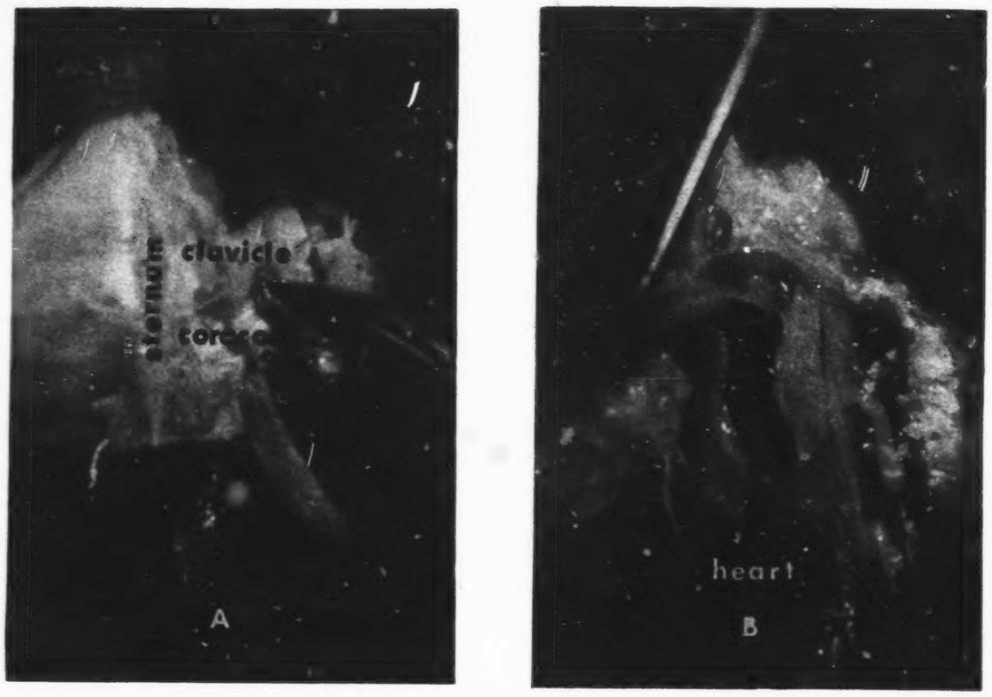

sternum
clavicle
corac
A
heart
B

Fig. 11.3
A. Cutting through the left clavicle and coracoid.
B. Lifting out the sternum and muscles.

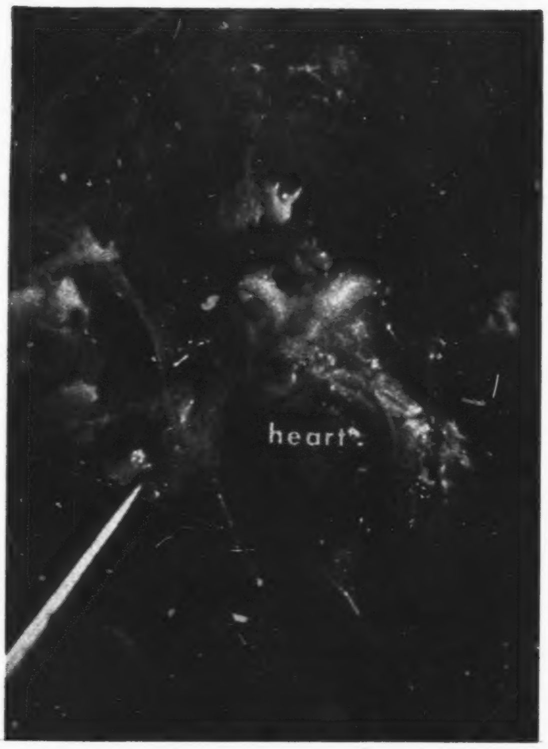


Fig. 11.4 Removing other parts of pectoral girdle.

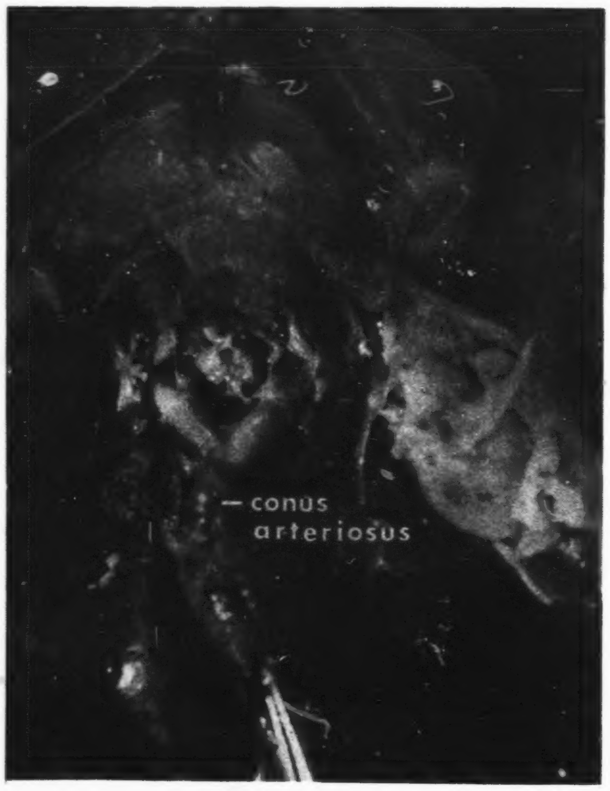

conus arteriosus

Fig. 11.5 The conus arteriosus, branching into two ventral aortas.

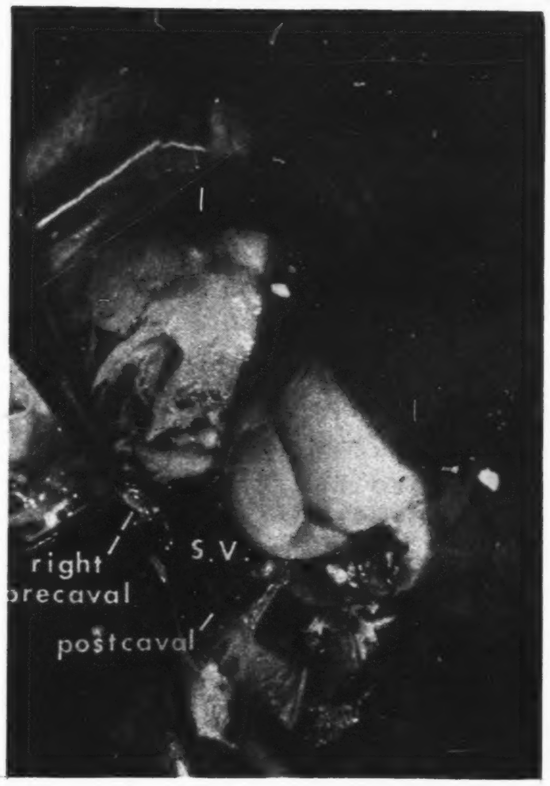

right
recaval
S.V.
postcaval

Fig. 11.6 Watch so you do not destroy these large veins. Heart lifted; S. V. = sinus venosus.

III. THE HEART

A. COVERING

Find the very thin, transparent pericardial sac. Remove it very carefully, both from the heart and from the large blood vessels that are connected to the heart.

B. PARTS OF THE HEART

1. Ventricle, the most posterior portion, cone-shaped, very thick-walled.

2. Two atria, anterior to the ventricle, thinner-walled. Atria are often mistakenly referred to as auricles.

3. Conus arteriosus, a large vessel that extends forward from the ventricle between the two atria. All the blood that leaves the heart passes through the conus arteriosus, just as in the lamprey. It is a thick-walled vessel, and divides into two branches just anterior to the heart. (see Fig.11.5) Is it proper to refer to this vessel as an artery? Why?

4. Sinus venosus. Lift the heart by the tip of the ventricle and identify a sac with thin translucent walls, through which you can probably see colored latex: this is the sinus venosus. You may have broken its thin walls when you took off the pericardial sac. The sinus venosus appears to be a dorsal posterior continuation of the right atrium, but the two are separated by a white line. Three large veins enter the sinus venosus, which you will need to know next week. They may not be injected in your specimen, so look for them carefully, and avoid breaking them: the one from the posterior end is the postcaval vein, and the two from the anterior-lateral sides are the right and left precaval veins. (See Fig. 11.6)

C. ROUTE OF BLOOD THROUGH THE HEART

From the lungs, blood returns to the left atrium via four tiny pulmonary veins which are not injected and which you probably will not see. From all other parts of the body, blood returns to the sinus venosus which then empties into the right atrium. Blood from both atria is pumped into the ventricle, mixed together, and is forced from the ventricle out through the conus arteriosus, supplying all parts of the body including the lungs. (This system is different in humans, where there are two ventricles; one of which pumps blood to the lungs only.)

IV. THE ARTERIES

Use care in dissecting this system. Remeber that there are often veins which run parallel to, or in the same area as, the arteries; and these veins should not be spoiled. Suggestion: In record keeping, try sketching each area separately, as you dissect it out, and then make on large sketch from the several small ones.

Find again the conus arteriosus. It divides near the anterior borders of the atria into two branches, the right ventral aorta and the left ventral aorta. Each ventral aorta immediately divides again into three aortic arches (called "arches" because they are curved like arches).

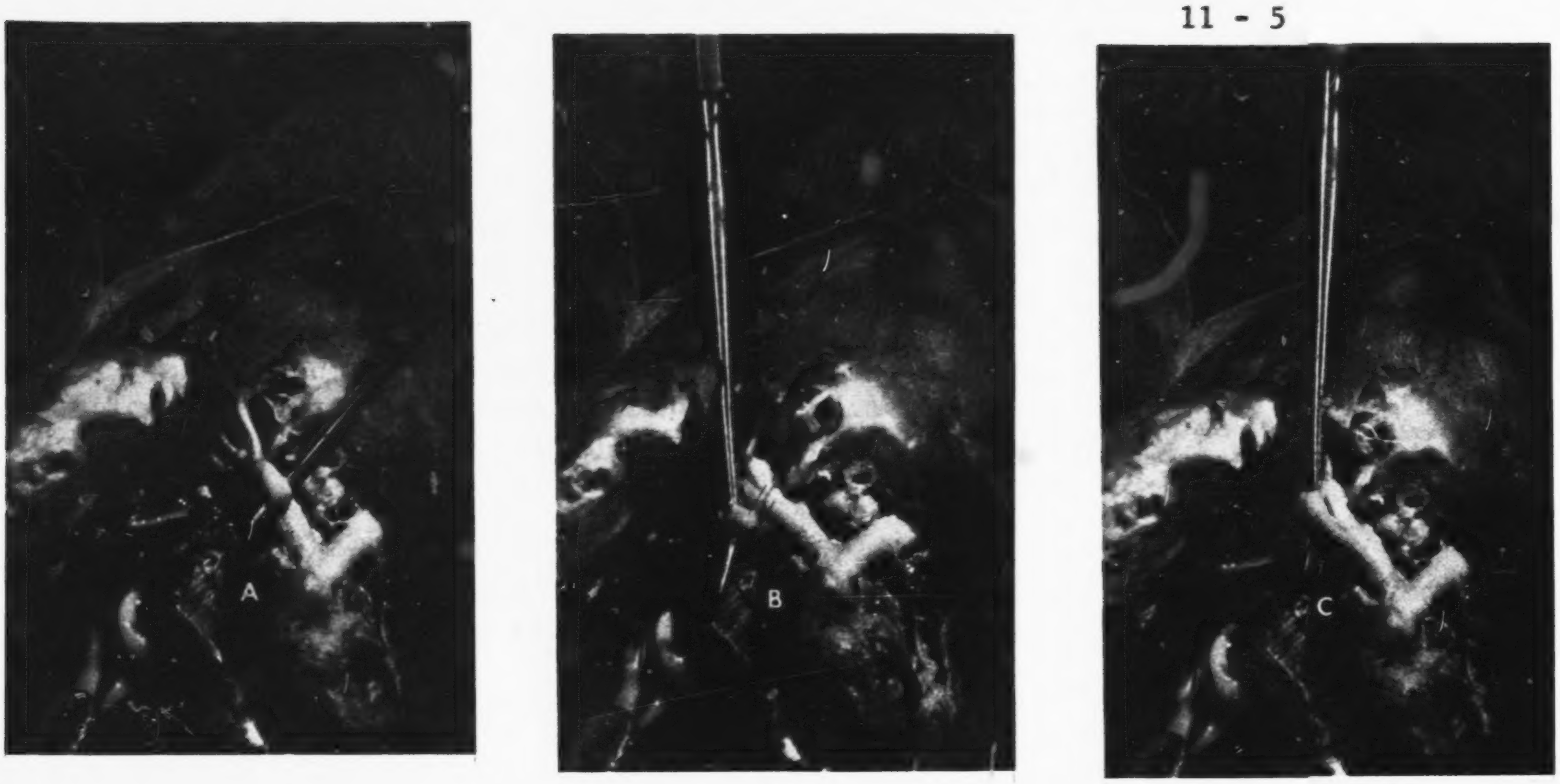


Fig. 11.7 *The three arches, all branches of the right ventral aorta.*
A: The carotid arch. *B: The pulmo-cutaneous arch.* *C: The systemic arch.*

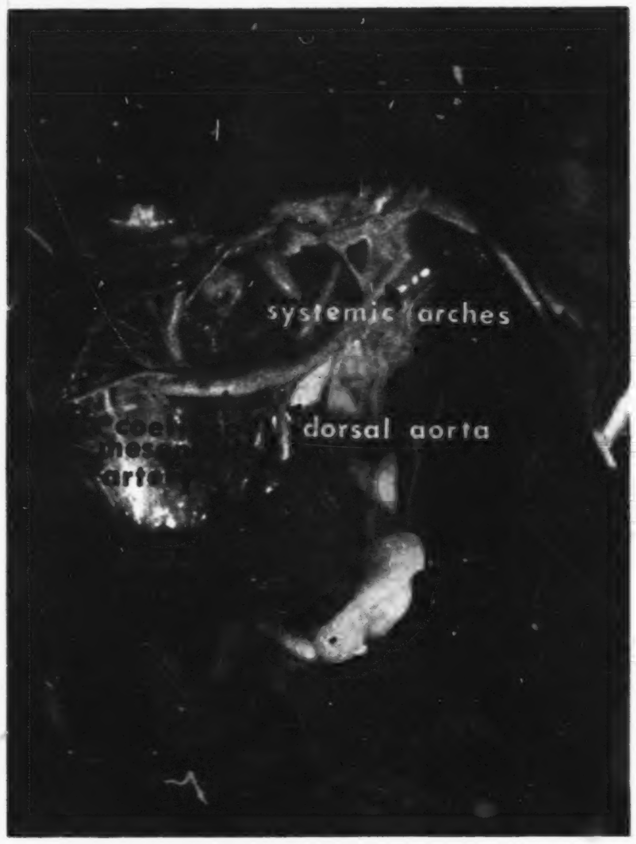

systemic arches
dorsal aorta
coel
mes
arte

Fig. 11.8 *Union of right and left systemic arches, as seen from the (frog's) left side, with digestive organs pulled to the right.*

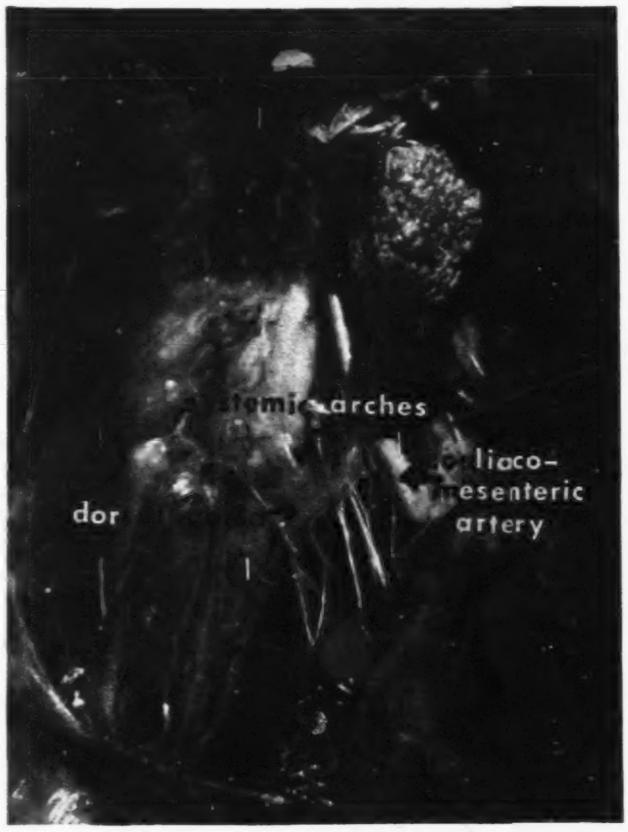

stemic arches
dor
liaco-
esenteric
artery

Fig. 11.9 *Union of right and left systemic arches, as seen from the (frog's) right side, with digestive organs pulled to the left. Notice the many branches.*

A. THE AORTIC ARCHES (See Fig. 11.7)

1. The carotid arch, the most anterior, which passes forward. Its two branches are:

    a) The external carotid artery, the smaller of the two, closest to the midline of the frog. Supplies the floor of the mouth and tongue. Remove the skin from the lower jaw and trace its branches.

    b) The internal carotid artery, the larger branch supplying the brain and the roof and sides of the mouth. Near the point of branching of internal and external carotids is an enlargement, the so-called carotid gland. Do not attempt to trace the internal carotid after it enters the muscles of the head region.

2. The pulmo-cutaneous arch, the most posterior branch of the ventral aorta. It divides into two vessels:

    a) The pulmonary artery, which is short and carries blood to the lungs.

    b) The cutaneous artery, which passes laterally and forward and disappears in front of the shoulder. Slit the skin on the dorsal surface and find the cutaneous artery emerging just anterior to the suprascapula. It supplies blood to the skin and to the side of the head.

3. The systemic arch, the middle branch of the ventral aorta. The systemic arches on each side of the frog supply blood to all the posterior parts of the body, including the organs of the coèlom and legs. They are bilaterally symmetrical, so you need dissect only one; but do the one thoroughly. Follow a systemic arch from its origin, dissecting away tissue as necessary but leaving veins in place. It passes laterally and dorsally, then turns posteriorly and goes back toward the midline of the body. The systemic arches from each side of the body meet again in the region just dorsal to the liver, to form the dorsal aorta. The following branches are given off before the dorsal aorta is formed---you should find them all:

    a) The laryngeal artery, branching off from the most anterior part of the systemic arch, and passing under the carotid gland, to the larynx.

    b) The esophageal arteries (sometimes one, sometimes two, from each systemic arch), passing to the dorsal surface of the esophagus.

    c) The occipito-vertebral artery, passing anteriorly along the side of the vertebral column, and giving off branches to the muscles of the body wall, the spinal cord, the sides of the head and jaws, and the brain.

    d) The subclavian artery, the largest branch of the systemic arch. It receives its name from lying under the clavicle, which you have removed. It carries blood to the shoulder region and the arm. In the arm it becomes the brachial artery.

B. THE DORSAL AORTA (See Figures 11.8 and 11.9)

Trace it posteriorly from its origin, which is the union of the two systemic arches. As it goes back, it gives off the following branches:

1. The coeliaco-mesenteric artery, which branches off the dorsal aorta at its very beginning, and passes ventrally to the organs of the coelom. It divides into two major branches:

   a) The coeliac artery to stomach, liver, pancreas and gall bladder.
   b) The anterior mesenteric artery to small intestine and anterior part of the large intestine, and spleen.

2. The urogenital arteries; paired arteries leaving the dorsal aorta laterally, taking blood to the kidneys, fat bodies and either ovaries or testes. Count and record the number of pairs of urogenital arteries in your frog, and compare with the findings of your colleagues in the lab.

3. The lumbar arteries, paired arteries to the dorsal body wall.

4. The posterior mesenteric artery, missing in many bullfrogs. If present, it is a single, small, median artery that supplies blood to the posterior part of the large intestine; if absent, then the posterior part of the large intestine is supplied with blood by either branches of the anterior mesenteric artery or branches of the iliac arteries.

5. The iliac arteries, a pair of arteries into which the dorsal aorta divides posteriorly (approximately in the region of the small intestine). The iliac arteries supply blood to the legs and to the urinary bladder. Trace the iliac artery into on leg, finding its major branches:

   a) The sciatic artery, supplying the dorsal part of the hip region.
   b) The femoral artery, which follows the femur closely.

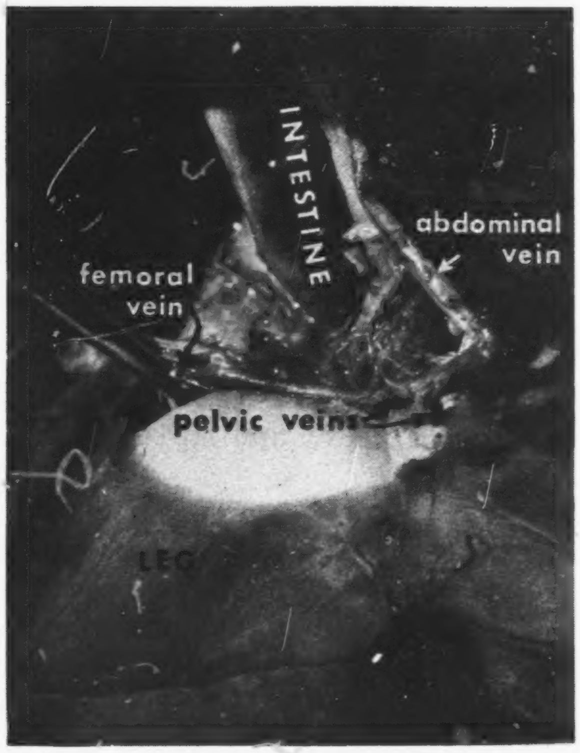

INTESTINE
abdominal vein
femoral vein
pelvic veins
LEG

Fig. 12.1 Lower abdominal cavity, showing branches of femoral vein. Abdominal vein is pulled toward right.

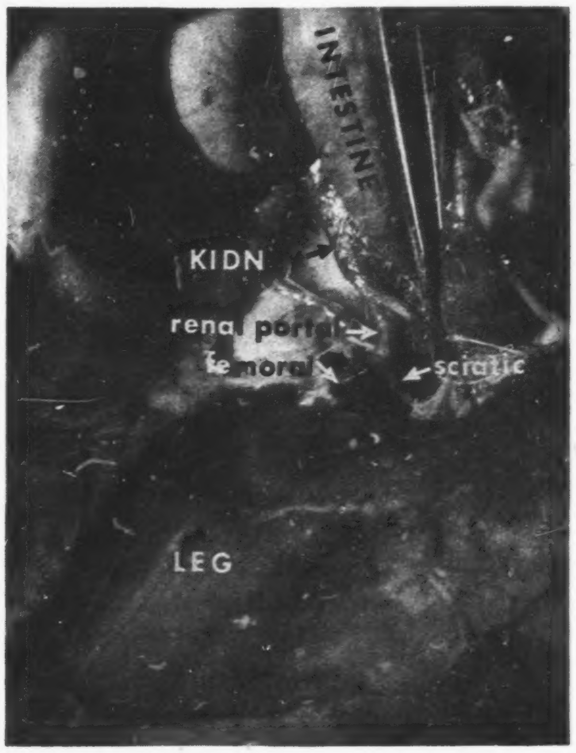

INTESTINE
KIDN
renal portal
femoral
sciatic
LEG

Fig. 12.2 Union of femoral and sciatic veins to form the renal portal vein.

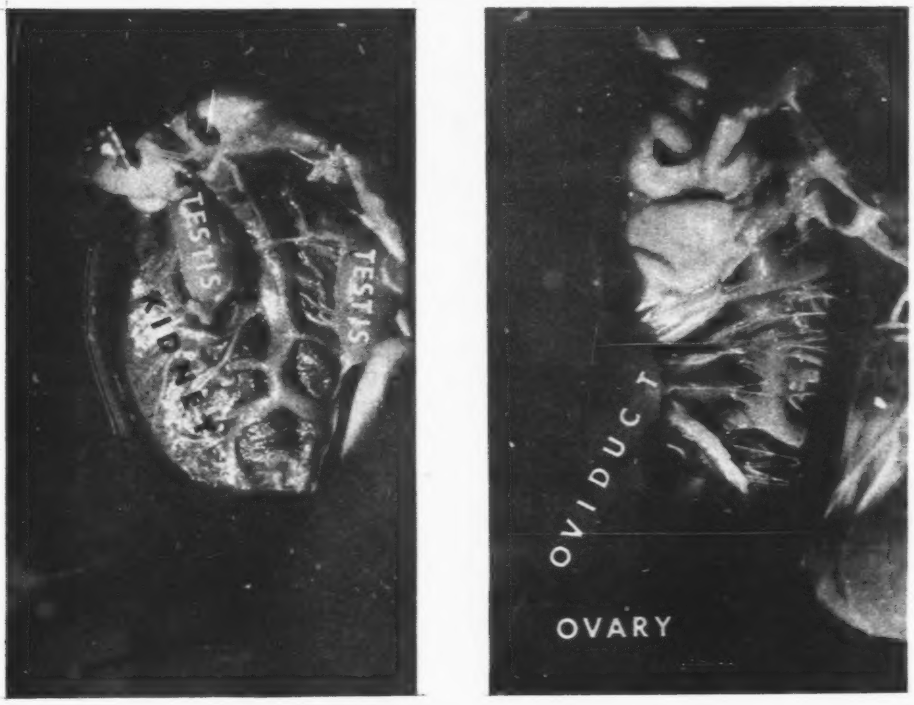

TESTIS
TESTIS
KIDNEY
OVIDUCT
OVARY

Fig. 12.3
Male (A) and female (B) renal veins, forming between the kidneys.

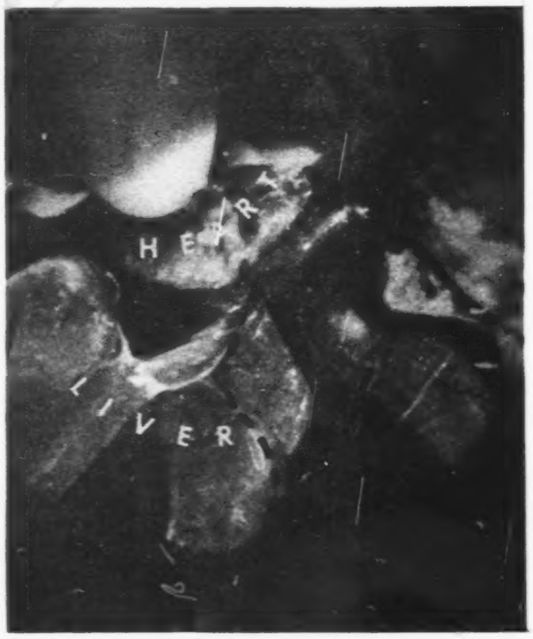

HEART
LIVER

Fig. 12.4 Route of postcaval through the liver (dashed line). Ventral view, but liver and ventricle lifted and tilted toward the left.

LABORATORY WEEK 12:
VEINS OF A BULLFROG

In dissecting for the venous system, we will begin out in the peripheral regions and trace the blood back to the heart. Thus we will leave until the last the difficult and variable large veins around the heart. The entire venous system is more variable than the arterial system, and is more difficult to find because often parts of it are not injected with latex at all. Note: this system is bilaterally symmetrical, so if you cannot find a particular vein on one side of the body, try the other side.

I. BLOOD RETURNING FROM THE LEG AND HIP REGIONS

Find the femoral vein, on the dorsal side of the femur, not far from the femoral artery. Blood in this vessel flows anteriorly, carrying blood back toward the heart. Trace this vessel forward, cutting through muscle and connective tissue as necessary. In the region where it enters the abdominal cavity, it gives off a branch, the pelvic vein (see Fig. 12.1). This vein passes medially across the floor of the abdominal cavity to the midline, where it meets the pelvic vein from the other side of the body. The two veins form the abdominal vein, which you first discovered when performing the preliminary dissection.

After giving off the pelvic vein, the femoral vein passes forward into the abdominal cavity and receives a branch, the sciatic vein, from the region of the lower back. Where the sciatic vein joins it, the femoral vein changes names and becomes the renal portal vein (see Fig. 12.2). Review, so that you are sure you know what a portal vein is. Trace the renal portal vein forward and find it running along the lateral border of the kidney. It enters the kidney through several small branches, which break up into capillaries inside the substance of the kidney. Find the dorso-lumbar veins (sometimes one, sometimes several) which carry blood from the muscles of the back into the renal portal vein.

II. BLOOD RETURNING FROM THE KIDNEYS AND REPRODUCTIVE ORGANS

Blood from the kidney capillaries is picked up by several small veins, the renal veins. These can be found extending from the medial border of each kidney. The renal veins from the two kidneys all run together at the midline and form a large vein, the postcaval vein. This is the largest vein in the region. Trace it forward. Note that there are several veins that carry blood into the postcaval vein. Find especially those from the fat bodies, the adipose veins. If your specimen is a male, find the veins from the testes, the spermatic veins. If a female, find the veins from the ovaries, the ovarian veins, and those from the oviducts, called oviduccal veins (see Fig. 12.3).

Trace the postcaval vein forward until it disappears into the substance of the liver. Do not attempt to trace it through the liver, but pick it up on the dorsal side of the liver. Find where it empties into the sinus venosus (see Fig. 12.4).

III. BLOOD RETURNING FROM THE ABDOMINAL REGION

Blood in this region returns through the hepatic portal system, the second portal system in the frog's body. (This one is found in humans also, but the renal portal system is not.) Two large veins contribute to this system:

1. The abdominal vein has already been identified in the mid-ventral line. It receives blood from the legs, urinary bladder and ventral body wall. It is formed in the pelvic region by the union of the right and left pelvic veins (see Fig. 12.1). Trace the abdominal vein anteriorly. At its anterior end, near the liver (or sometimes within the substance of the liver) it divides into right and left branches, one branch carrying blood to the right lobe of the liver, the other to the upper left lobe of the liver. Both branches meet with branches of the hepatic portal vein (see below).

2. The hepatic portal vein is a large but short vein, formed by the union of several veins bringing blood from the organs of the digestive system: from the stomach, the entire length of the small intestine, and the anterior end of the large intestine. In addition, blood is brought from the spleen. The hepatic portal vein passes anteriorly through the substance of the pancreas and then forks into two branches. One branch goes to the lower left lobe of the liver, and the other joins the abdominal vein just before it divides (see Fig. 12.5).

The two veins of the hepatic portal system carry blood into the substance of the liver, and there break up into sinusoids (like irregular capillaries). What is the function of the liver in relation to the hepatic portal system? Blood from these sinusoids is picked up by small veins leading to the right hepatic vein and left hepatic vein. These veins can be seen on the dorsal side of the liver, entering the postcaval vein just before it enters the sinus venosus (see Fig. 12.4).

IV. BLOOD RETURNING FROM THE SKIN AND MUSCLES OF THE BACK AND SIDES

Examine the flap of skin that was turned back when opening the body cavity. Look especially in the region of the armpit. Find a large vein that adheres to the skin, the cutaneous vein. Examine the muscle that lies just underneath the cutaneous vein, and find the muscular vein. These two join together (though you have probably destroyed their juction) to form the musculocutaneous vein (see Fig. 12.6). Find where this vein joins with the other veins in the shoulder region.

V. BLOOD RETURNING FROM THE ARM

Dissect the upper part of the arm and find the large vein that drains blood from it, the brachial vein. Trace it from the arm toward the heart. If it joins with the musculo-cutaneous vein, the resultant vein is referred to as the subclavian vein (see Fig. 12.7). These two veins may lead to the precaval vein independently, without joining together, and in that case there would be no subclavian vein. The subclavian vein is so named because it lies just dorsal to the clavicle, which you have removed.

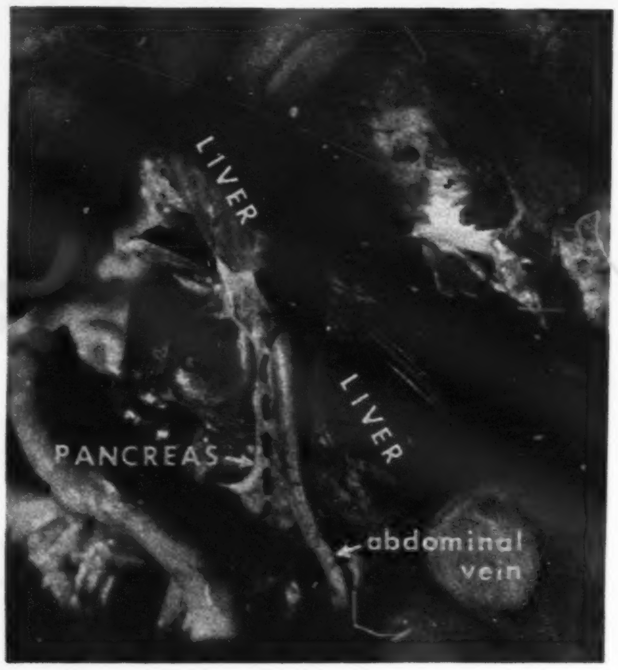

LIVER
LIVER
PANCREAS
abdominal vein

Fig. 12.5 Hepatic portal system. Hepatic portal vein follows dashed line through pancreas substance, into liver.

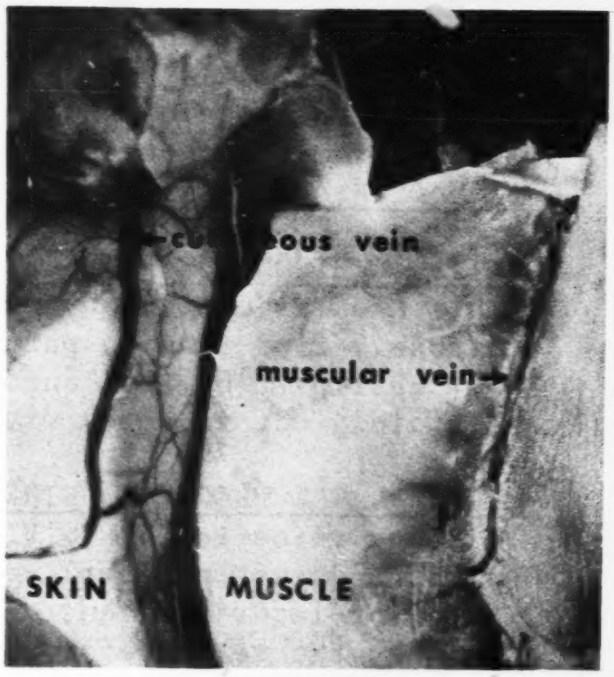

cu eous vein
muscular vein
SKIN
MUSCLE

Fig. 12.6 Dissected skin and muscle, showing location of cutaneous and muscular veins.

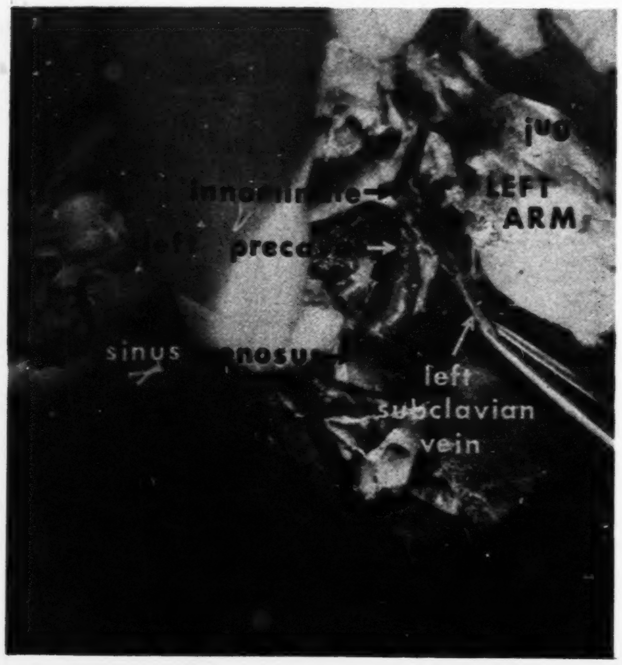

jug
innominate
LEFT ARM
left precaval
sinus venosus
left subclavian vein

Fig. 12.7 Location of subclavian vein. Ventricle is pulled aside.

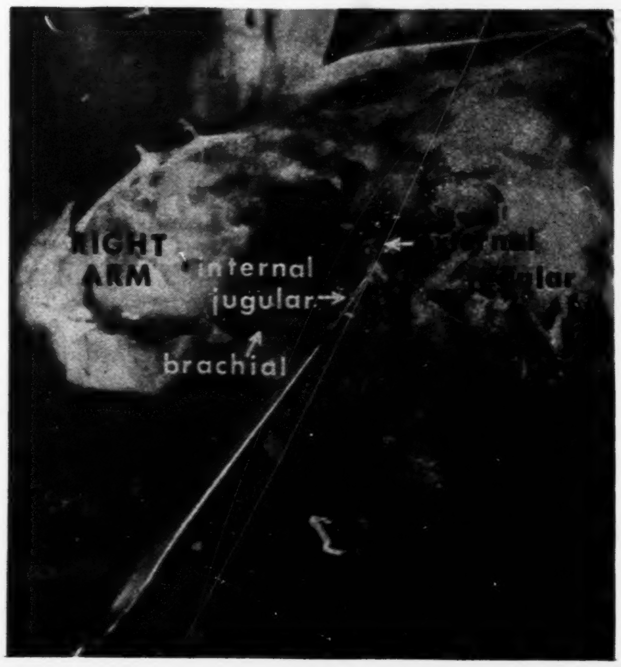

RIGHT ARM
internal jugular
brachial
internal jugular

Fig. 12.8 A case where all veins lead independently to precaval vein.

VI. BLOOD RETURNING FROM THE DORSAL HEAD AND BRAIN REGION

This region is drained by the internal jugular vein (see Fig. 12.8). You will probably find this vessel running close to the internal carotid artery for at least part of its length. The internal jugular vein may lead directly to the precaval vein without joining with any other vessel, but more often it joins either with the brachial vein or with veins from the shoulder region; such a combined vein is called the innominate ("nameless") vein.

VII. BLOOD RETURNING FROM THE LOWER JAW, TONGUE AND THROAT REGIONS

This area is drained by the external jugular vein, which you will probably find running parallel to the external carotid artery for at least part of its length. The external jugular vein is almost certainly not injected with latex in your specimen (see Fig. 12.8).

VIII THE PRECAVAL VEINS

All the veins in IV, V, VI and VII above will lead eventually to the right and left precaval veins (see Fig. 12.8). Find these as thick anterior-lateral extensions of the sinus venosus. The various junctions made by the veins before they arrive at the precavals cannot be described here, because each frog is different in this region--your own frog may even be different on opposite sides. Make sure that you know how your own frog is arranged. All the veins so far described empty their blood into the sinus venosus and from there the blood goes into the right atrium.

IX. BLOOD RETURNING FROM THE LUNGS

This is the only blood that does not return to the sinus venosus. Instead, it is carried directly to the left atrium. Find these small veins, probably four in number, on the dorsal side of the left atrium, and trace them to the lungs. They are called pulmonary veins.

Make an appointment with your conference instructor to take the individual oral practical quiz. This should last about five minutes, and will cover the circulatory system, including all the material in Laboratory Weeks 11 and 12.

LABORATORY WEEK 13:
MICRO-MORPHOLOGY OF VERTEBRATE MESODERMAL DERIVATIVES

I. BACKGROUND

The connective tissues, including muscle, make up the greatest bulk of the body; and although they do not resemble each other in gross structure, many of them show surprising similarity under the microscope. In the developing embryo, they all arise from a generalized type of mesodermal tissue such as you find in II below, with the cells rather close together, but separated by a small amount of jelly-like inter-cellular matrix. In developing into different tissues, the cells of connective tissue proper secrete larger or smaller amounts of matrix around themselves, and this matrix may be liquid or fibrous or firm or bony, or a combination of these. The cells remain relatively undifferentiated

In the case of muscle, it is the cells themselves that become differentiated--lengthening and becoming elastic, bound together with fibers produced by other cells in the group.

A word about the slides provided for you: These slides have been selected carefully in order to show you the necessary details in each case. The tissues will not look "normal" -- the material has been made very thin, and then stained with special biological dyes (primarily hematoxylin) that will color certain parts and leave other parts clear or light-colored. For example, the nucleus of a cell appears very dark after staining, but in life it is almost colorless. In making the material thin enough to observe, two general methods have been followed:

1. Sectioning, where the various parts are in their normal position, and merely sliced very thin. Thickness about 10 microns.

2. Smearing or teasing, where the soft material is spread out thinly on the slide, thus taking the parts out of natural position but leaving the cells intact.

Of the two methods, sectioning is more difficult but more satisfactory for most tissues. Most of the slides that you will see are sectioned.

II. EMBRYONIC CONNECTIVE TISSUE.

Slide #26.-- Adjust the slide on the microscope stage under low power. Find a region on the slide where the material looks almost white; where the cells and fibers are very widely separated from each other (see Fig. 13.1).

A. THE WHOLE TISSUE UNDER LOW POWER

Have the laboratory assistant help you find the cells if you have difficulty, and notice the size and extent of their nuclei. The clear spaces on the slide were, in life, filled with a jelly-like matrix. In the matrix, between the cells, you will find very fine collagenous fibrils. These are condensed parts of the matrix, and are not parts of the cells.

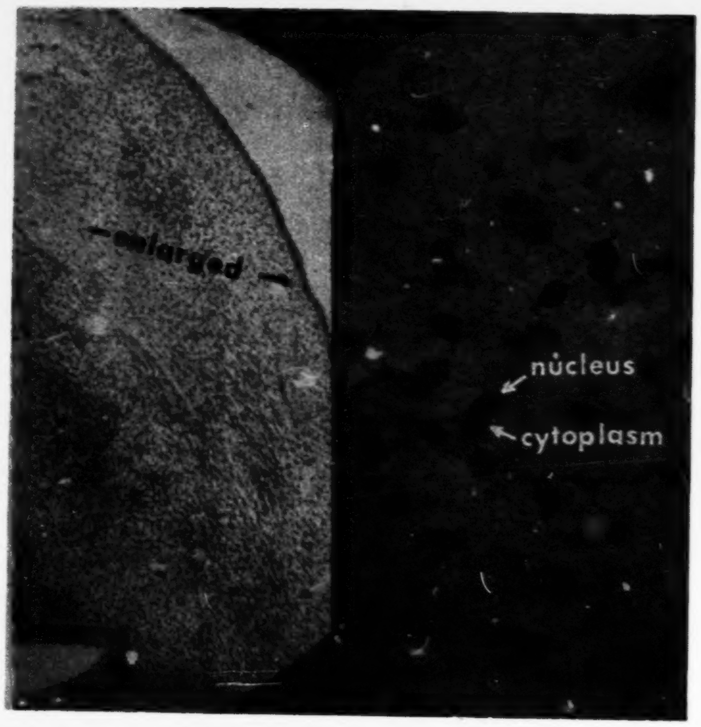


Fig. 13.1 Embryonic connective tissue, low power and high power.

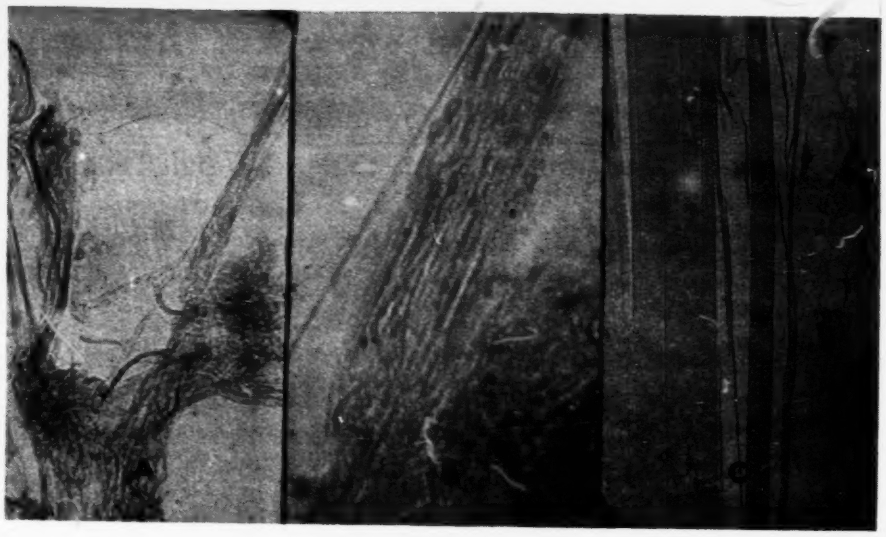


Fig. 13.2 White fibrous tissue. A is low power and teased; B is high power and teased; C is a low power longitudinal section of a tendon, as in the demonstration.

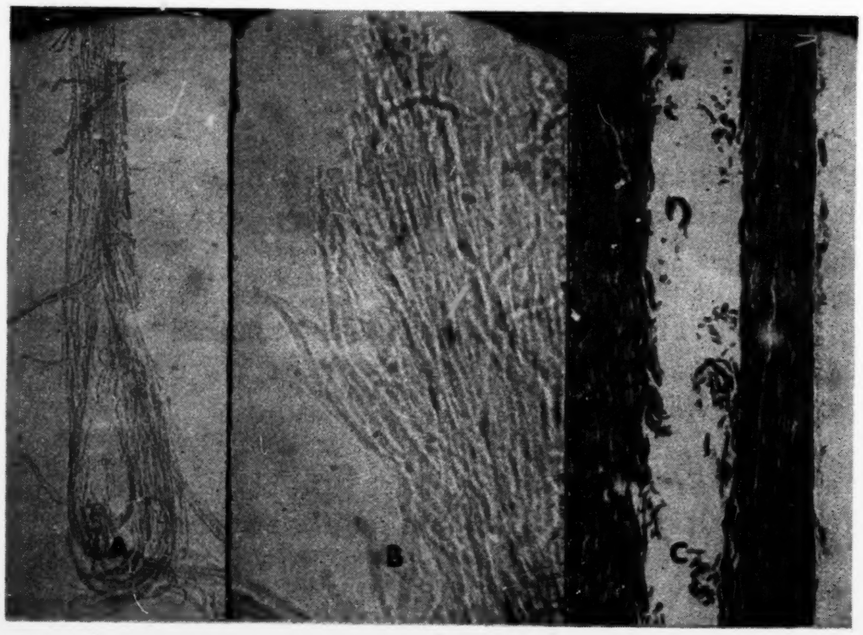


Fig. 13.3 Yellow elastic tissue. A is low power and teased; B is high power and teased; C is a low power longitudinal section of a ligament, in which the dark fibers are elastic.

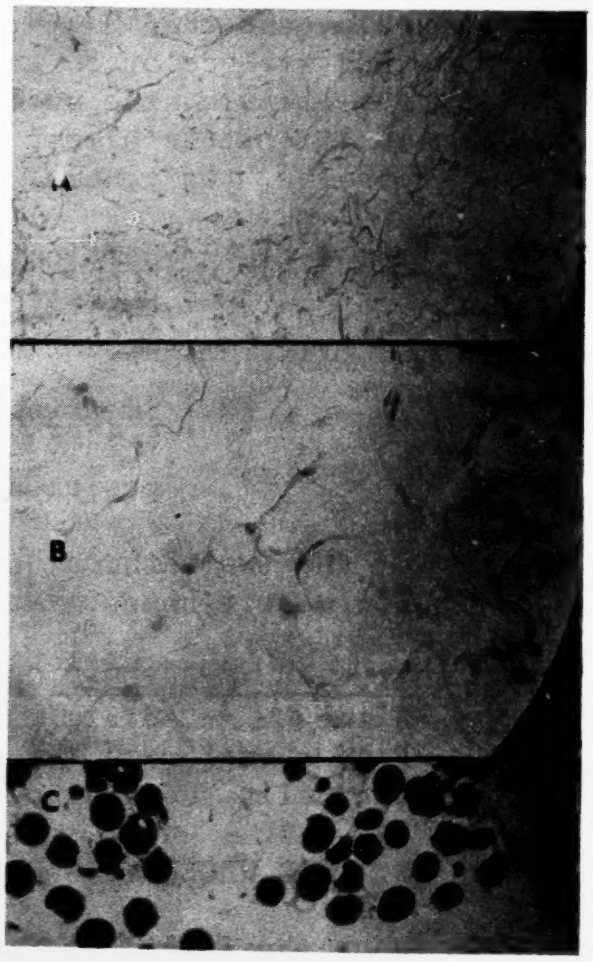


Fig. 13.4 Adipose tissue. A is low power; B is high power; C is a section through adipose tissue stained with osmic acid, in which the fat droplet is black.

B. THE TISSUE UNDER HIGH POWER

1. Cells -- Center one isolated cell. The cytoplasm is almost colorless, and you can distinguish the cell membrane in only a few places at best. You should be able to find occasional strands of cytoplasm extending from one cell to another. The cells are sometimes described as "stellate", or star-shaped. Compare these cells with those you scraped from the lining of your cheek, during laboratory week 1.

2. Fibrils -- Examine the collagenous fibrils with special care. See that in this embryonic tissue they are neither oriented in any one direction, nor gathered into groups to make large fibers. In older tissues, both orientation and gathering occur, and that will give special characteristics to those tissues. Collagenous fibrils, gathered in bundles, are collagenous fibers.

III. FIBROUS TISSUES

The slides provided in the slide box are smears, and the fibers have been teased apart with needles. The cells are not readily apparent -- we are mainly interested in the fibers that the cells have produced. Demonstration slides are also set up of the same tissues sectioned, in order to show the normal relationship of the cells and fibers.

A. WHITE FIBROUS TISSUE

Slide # 27. -- Tendons are made of this tissue. Under low power, find a place where the fibers are separated from each other (see Fig. 13.2). Find the collagenous fibers, and under high power see that they are made up of very tiny collagenous fibrils, such as in embryonic connective tissues. The fibers are all oriented, running in one direction, and the cells are irregularly spaced, crowded in between the fibers. Notice that there is very little space for them. Are all the fibers of the same thickness? All the fibers are of the collagenous type.

B. YELLOW ELASTIC TISSUE

Slide # 28. -- Some ligaments are made of this tissue. Examine as above, and compare with white fibrous tissue as to numbers and placement of cells and fibers (see Fig. 13.3). The tissue was not stained for collagenous fibers and fibrils, so although there are many of them, they are not easily seen. The fibers you see best are elastic fibers, which are yellow in life but lightly-stained here. Elastic fibers are intermediate in size between collagenous fibrils and collagenous fibers. You should be able to see that some elastic fibers branch, while collagneous ones do not. Notice especially that elastic fibers are not collections of fibrils, but are simply solid fibers.

IV. ADIPOSE TISSUE

Slide # 29. -- These slides are sections. Examine under both low and high power. In this tissue, the cells are swollen with a fat droplet, and the cells have become so crowded together that the matrix is almost obliterated. Notice that the nucleus has been pushed over to one side of the cell, next to the cell membrane. (see Fig. 13.4).

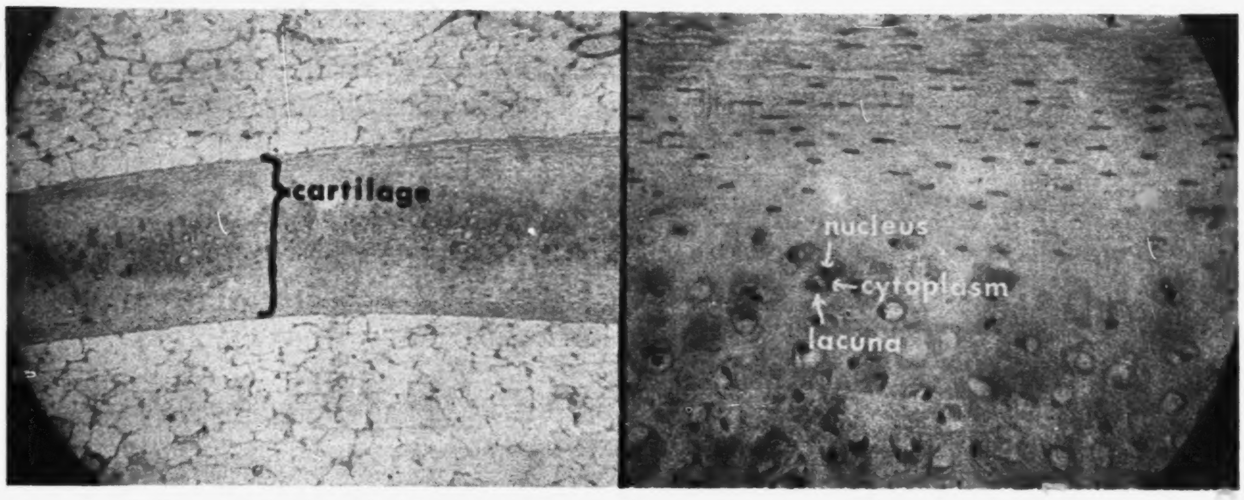

cartilage
nucleus
cytoplasm
lacuna

Fig. 13.5 Hyaline cartilage.

Low power High power

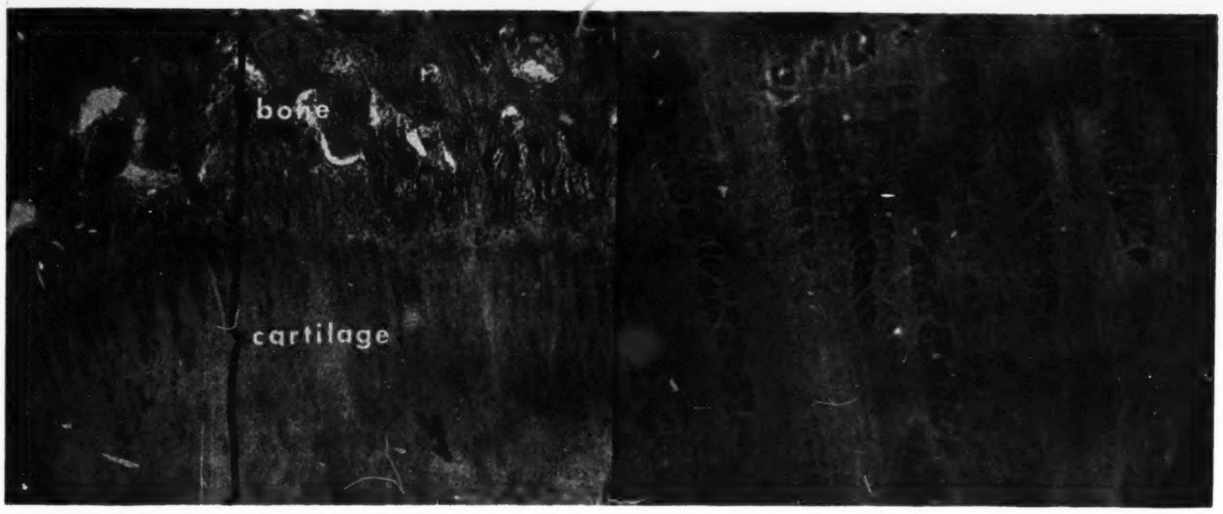

bone
cartilage

Fig. 13.6 Fibrocartilage.

Low power High power

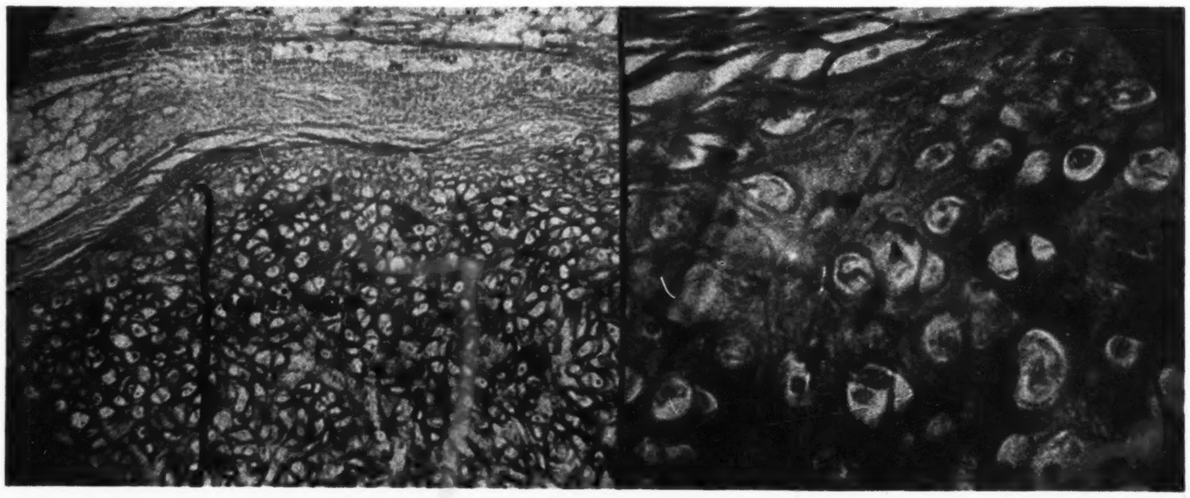


Fig. 13.7 Elastic cartilage.

Low power High power

Some of the sectioned cells are said to look like diamond rings, with the nucleus acting like the diamond. Can you find any collagenous fibrils in this tissue? Nearly any connective tissue can develop into adipose tissue, simply by adding fat to some or all of the cells.

V. CARTILAGE

In this connective tissue, the matrix of the original embryonic connective tissue (from which it developed) has become hardened or stiffened by the addition of protein materials. By secreting this firm matrix, the cells of the cartilage trap themselves in small spaces called lacunae. There are fibrils in the matrix of all cartilage, but in most cartilage they are invisible because they do not stain differently from the rest of the matrix. This is especially true of the hyaline ("clear") cartilage. Some cartilage contains collagenous fibers in the matrix, which are visible, and this is called fibrocartilage. Other cartilage contains elastic fibers in the matrix, and this is called elastic cartilage.

A. HYALINE CARTILAGE

Slide #30. -- Most of this tissue is made up of matrix, with the cells scattered ore or less at random through it. The cells in the slide have partially pulled away from the walls of the lacunae, leaving some empty space (see Fig 13.5). You may find some lacunae with two cells; or two lacunae close together with flattened adjacent sides; indicating that cell division has recently taken place. Where can you see fibrils or fibers? What kind of tissue do you find surrounding the cartilage on the slide?

B. FIBROCARTILAGE

Slide #31. -- This is an uncommon kind of cartilage, mainly found inside vertebrae. Under low power, find the bone (bright red) in the slide. In the bone, look for purple areas, which are fibrocartilage (see Fig. 13.6). Examine these with high power, and carefully compare with hyaline cartilage.

C. ELASTIC CARTILAGE

Slide #32. -- This is found in several parts of the body, such as the nose and ear. Examine the slide and find the typical cartilage structures, plus the elastic fibers (see Fig. 13.7).

VI. BONE.

In bone, the matrix is hardened as in cartilage by the addition of protein materials, and in addition there are crystals of calcium and phosphorus salts that further harden the tissue. As in cartilage, the cells are trapped by their own secretions, and must live in lacunae. But unlike cartilage, there are blood vessels and nerves that run throughout a bone, bringing nourishment and oxygen, carrying away wastes, carrying sensations. These vessels run lengthwise in a long bone, inside spaces called haversian canals. A vein, an artery and a nerve are usually found together in one canal. The bone cells are found in concentric layers around the haversian canal. Since the diffusion of food, oxygen, etc. would be very slow through bone, the bone substance is penetrated everywhere by extremely fine openings called canaliculi, which radiate out from the lacunae and which, in life, contain tiny extensions of the bone cells.

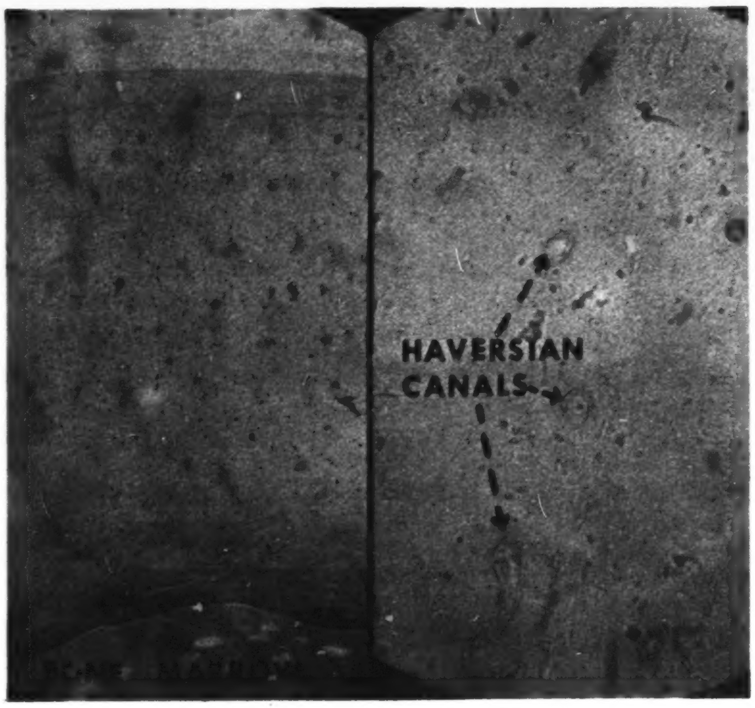

HAVERSIAN
CANALS
BONE MARROW

Fig. 13.8 Decalcified bone of frog.
Low power High power

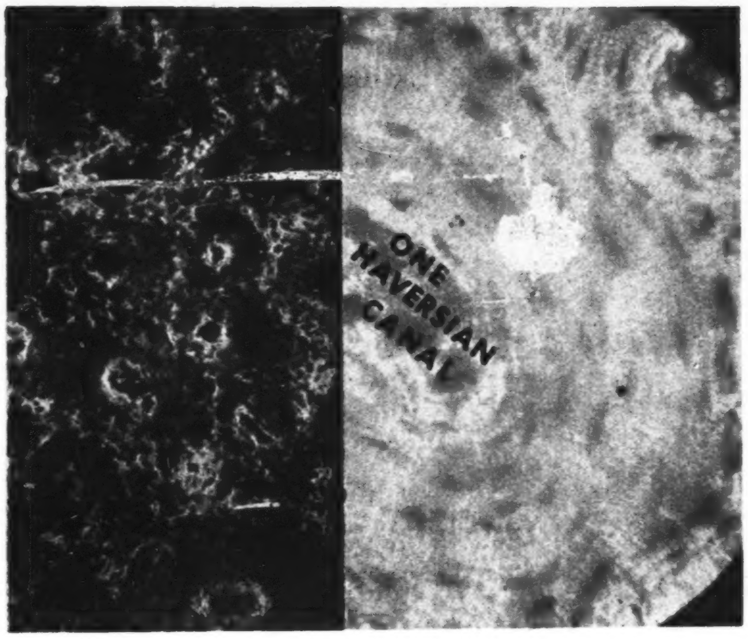

ONE
HAVERSIAN
CANAL

Fig. 13.9 Dry bone section, human.
Low power High power

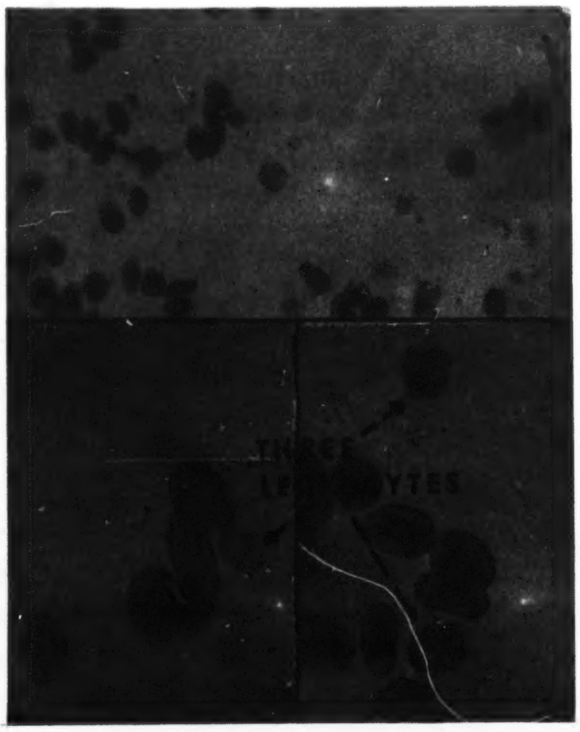

THREE
LEUCOCYTES

Fig. 13.10 Frog blood, stained.
Low power above, high power below.

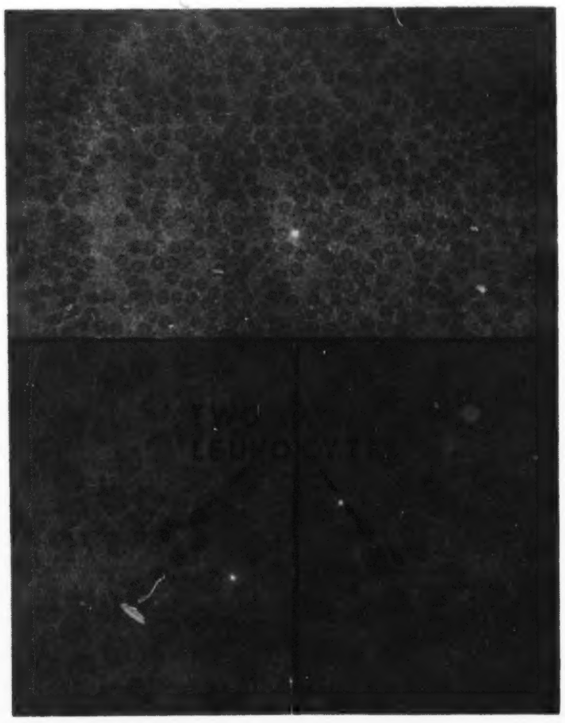

TWO
LEUCOCYTES

Fig. 13.11 Human blood, stained.
Low power above, high power below.

A. DECALCIFIED BONE

Slide #33. -- This is a cross-section through a long bone, prepared by first soaking the bone in weak nitric acid for several days to dissolve out the calcium and phosphorus salts, and then sectioning. The decalcification removes on the salts, and leaves behind all the cells, matrix and fibers. Examine the slide under low power, and compare what you see with the fibrous connective tissue (see Fig. 13.8). Fibrils? Fibers? Haversian canals?

B. DRY BONE, GROUND THIN

Slide #34. -- A slice of a long bone was sawed off, then polished down to a thin section, and mounted dry. Wherever there is air in the slide, the section appear dark. There is nothing in this bone but the calcium and phosphorus salts, which had been decalcified out in the slide above. This slide is a kind of "negative" of decalcified bone (see Fig. 13.9). No cells, fibers or blood vessels remain, and the spaces they occupied are filled with air. See that the matrix of salts has been arranged in concentric layers called lamellae, around the tube-like haversian canals, which in cross-section look like large black dots. Between the layers of lamellae are layers of lacunae, with their many extremely thin radiating canals, the canaliculi. One haversian canal with its surrounding lacunae, canaliculi and lamellae is called a haversian system.

VII. BLOOD

The matrix of this tissue is a rather sticky fluid, the plasma, in which the cells float freely. Under normal conditions there are no fibrils nor fibers in plasma: they only appear when blood clots, and then they are different from the collagenous fibrils you have seen. The cells of the blood are of two different kinds:

a) The very numerous erythrocytes, having a red-reflecting pigment (hemoglobin) in the cytoplasm.
b) The relatively rare leukocytes, which have no red pigment.

A. FROG BLOOD

Slide #35 . -- Examine the prepared smear of frog blood (see Fig. 13.10). These slides have been stained and therefore all the nuclei will be violet or purple in color. The erythrocytes all have nuclei which are quite large and oval in shape. (In human blood, erythrocytes have no nuclei.) You will have to search for leukocytes. These are of several different shapes, but will appear different from erythrocytes. How many different kinds can you find? Examine under high power.

B. HUMAN BLOOD

Use either Slide # 36 or the slide obtained from IX A-5 below. Identify the erythrocytes, and examine as many kinds of leukocytes as you can find (see Fig. 13.11). Notice the striking similarity between the leukocytes of frog and human, but the great difference in the erythrocytes. Human erythrocytes have no nuclei, and are shaped like bi-concave discs.

VIII. MUSCULAR TISSUES

All three slides of muscle have been prepared as smears -- teasing apart the different fibers and then staining them. They will therefore not be arranged normally, but you will be able to see whole cells rather than slices. Demonstration slides are also set up of the same tissues sectioned, in order to show the normal relationships of the cells.

All muscle cells are longer than they are wide, and are able to "contract" (this means become shorter and thicker, not decrease in total volume). The muscle fibers in a muscle are bound together and interwoven with fibrils of connective tissue. Aside from these points of similarity, the three types of muscle described below are very different from each other.

A. SMOOTH MUSCLE OR INVOLUNTARY MUSCLE

Slide # 37. -- This is the kind of muscle found in the digestive system, urinary system and other places where the contraction must be slow and prolonged, and is governed by involuntary nervous reactions. Each fiber is an individual cell with a separate nucleus. Look at a region where the fibers are widely separated, and find the location of the nucleus. Under high power, look for very fine longitudinal lines within the cells: the fibrillae. It is these fibrillae that do the work of contracting the muscle cell. (see Fig. 13.12).

B. STRIATED MUSCLE OR VOLUNTARY MUSCLE

Slide # 38. -- This is the kind of muscle found attached to bones, and is therefore sometimes called "skeletal muscle". The contraction must be rapid and or short duration, and is governed by voluntary nervous reactions. Each fiber is made up of many cells, without cell membranes between them. Each fiber contains several nuclei just under the sarcolemma (= the muscle fiber's cell membrane). In the sarcoplasm (cytoplasm) find the tiny longitudinal fibrillae, which make the indistinct longitudinal markings. There are swellings on the fibrillae, which show up as very distinct transverse striations which give the muscle type its name. (see Fig. 13.13).

C. CARDIAC MUSCLE OR HEART MUSCLE

Slide # 39. -- Notice that the fibers that make up this muscle are many-nucleated and striated, somewhat like striated muscle. But the individual fibers seem to run into each other at random, making the entire tissue one large mass of protoplasm. This run-together situation is called a syncytium. Scattered throughout the tissue you will find intercalated discs, which look as though they might be cross-walls dividing the cells from each other, but are not. These discs are points of attachment of the fibrillae, and their function is not well understood. (see Fig. 13.14).

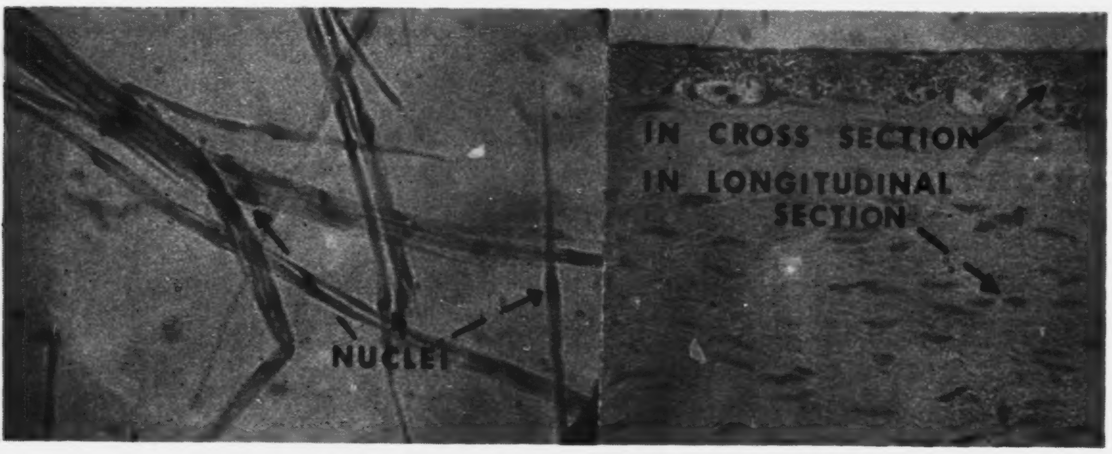

NUCLEI
IN CROSS SECTION
IN LONGITUDINAL SECTION

Fig. 13.12 Smooth muscle, high power.

Teased *Sectioned*

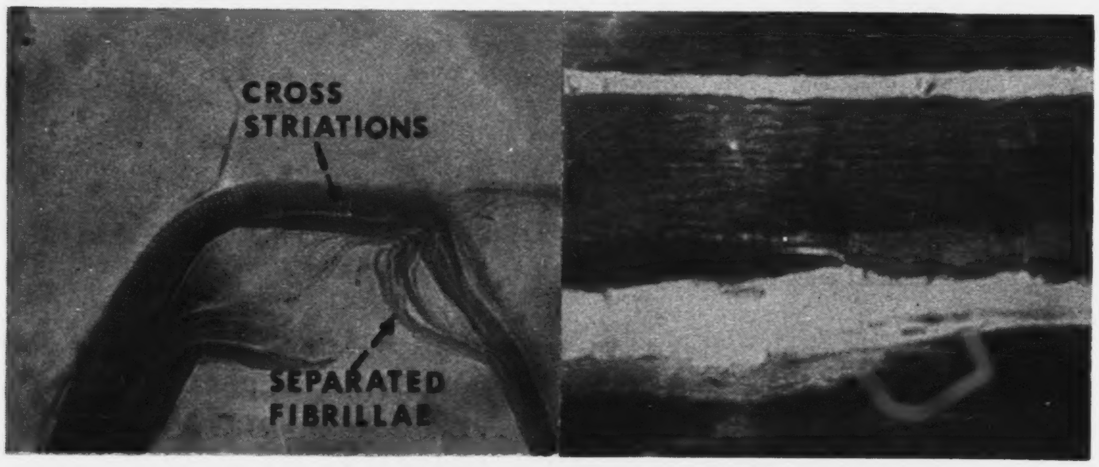

CROSS STRIATIONS
SEPARATED FIBRILLAE

Fig. 13.13 Striated muscle, high power.

Teased *Sectioned*

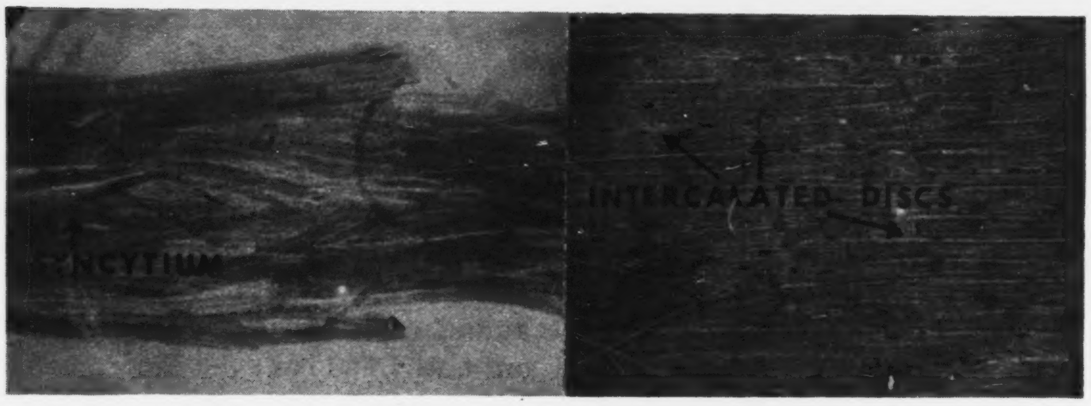

SYNCYTIUM
INTERCALATED DISCS

Fig. 13.14 Cardiac muscle, high power.

Teased *Sectioned*

IX. OPTIONAL PROCEDURE WITH HUMAN BLOOD

A. SLIDE PREPARATION

Clean six slides with 70% ethanol, to remove all oil from them. Let them dry in air. Mark two of them with L for living blood, and four of them with S for smear. Put a drop of 0.8% NaCl solution on each of the L slides.

B. OBTAINING THE BLOOD

1. Clean a finger thoroughly with 70% ethanol, and dry it by swinging your arm vigorously back and forth from the shoulder.

2. Open a sterile disposable lancet and use it at once, firmly, on the clean finger. Do not wait for things to get contaminated. Throw the lancet away immediately, and do not use it again for any purpose.

3. After putting the blood on the slides (see below), wipe the finger with 70% ethanol.

C. USING THE BLOOD

1. L slides. Add a drop of blood to the salt solution and cover with a coverglass. These slides may then stand for five to ten minutes while you make the smears.

2. S slides. Make on of these slides at a time, working rapidly, and keep trying until you obtain a perfect smear. The most common errors here are using too large a drop of blood, and letting it clot before smearing.

    a. Put a drop of blood near the end of the slide as in Fig. 13.15.
    b. Slant another slide over the first one, and pull it toward the drop of blood until it touches and the drop spreads, as in Fig. 13.16.
    c. Push the second slide rapidly toward the opposite end of the first one, as in Fig. 13.17.
    d. The pattern you want will probably look somewhat like a candle flame, with the smear thinning to nothing near the middle of the slide as in Fig. 13.18. If it does not, the drop was too large, and you should try again.
    e. Make more than one perfect smear, in case of failure in making the Wright's stain. Let the smears dry in the air. They may now stand indefinitely, while you observe the living blood on the L slides.

D. OBSERVATION OF LIVING BLOOD

Study the slide under low and high power. You should be able to observe the following:

1. Erythrocytes. These are the most numerous cells. What color are they? Does this surprise you? Do they all appear the same size and shape?

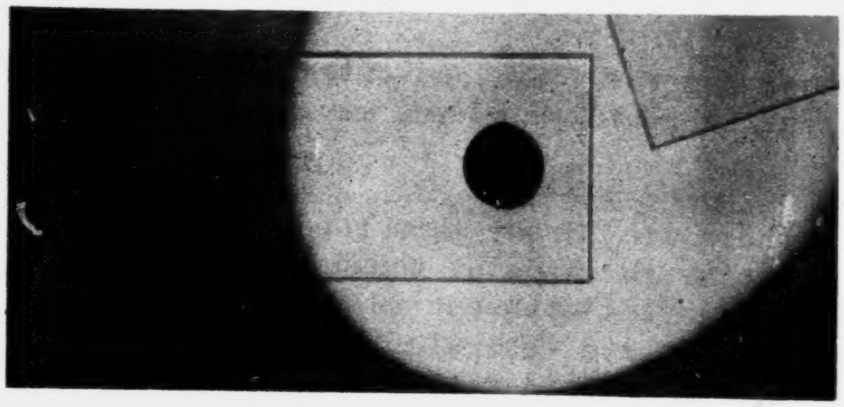


Fig. 13.15 A drop of blood near end of slide.

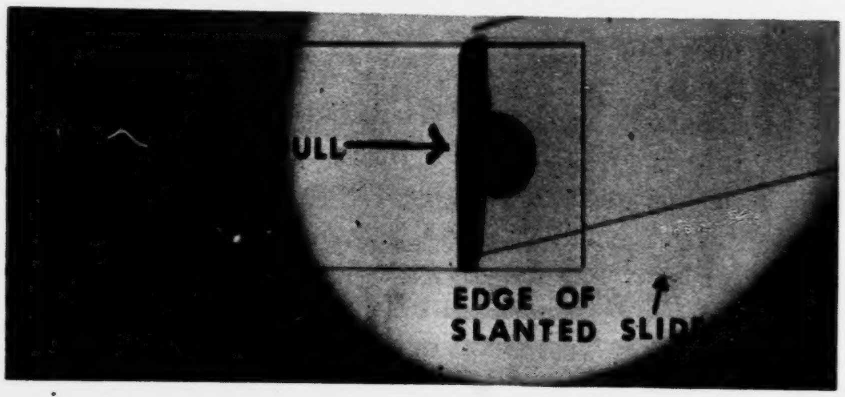

ULL
EDGE OF
SLANTED SLID

Fig. 13.16 Slide slanted, pulled to drop of blood.

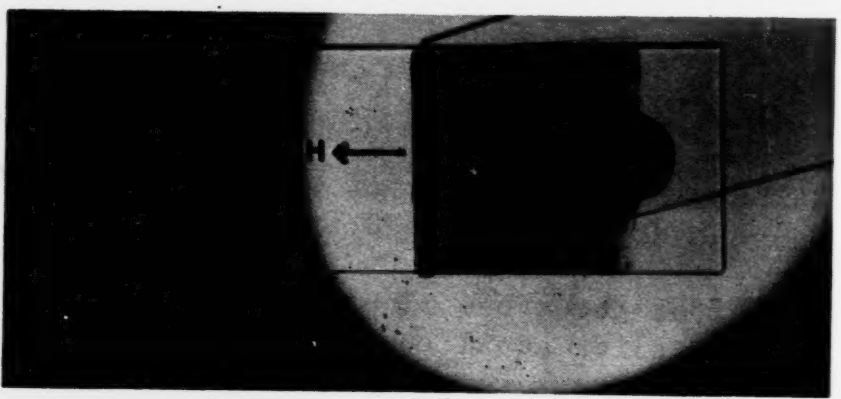


Fig. 13.17 Slanted slide pushed halfway.

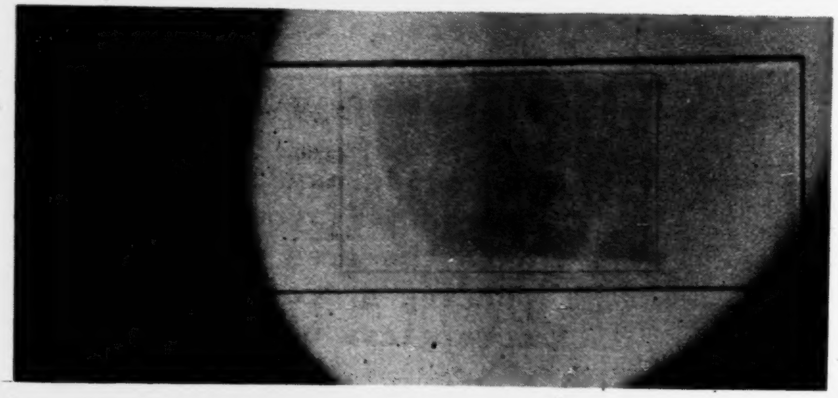


Fig. 13.18 Finished slide, after staining, showing smear thinning to nothing.

2. Blood platelets. These are very tiny, colorless, irregularly shaped. They disintegrate rapidly, so you may not see any at all.

3. Leukocytes. Most of these are larger than the erythrocytes, and may be either irregular in shape or rounded. Can you see the nuclei? Are there granules apparent in the cytoplasm? If you watch some of these that are irregularly-shaped, you may be able to see them undergoing ameboid motion, particularly after the blood has stood for ten minutes.

4. Rouleaux formation. This refers to the coin-like stacks into which rocytes become arranged as a normal thing.

5. Crenated corpuscles. These are the erythrocytes with scalloped margins, produced by osmotic differences between the medium and the interior of the cells. Does crenation mean that the solution was hypotonic or hypertonic?

6. Hemolysis. When you have finished observing the above, add a drop of distilled water to the edge of the coverglass and draw it through with a small piece of toweling. Watch the change in shape of the erythrocytes as they come into contact with the water. The cells swell and burst, leaving "ghosts" of their former shapes. This lysing of blood cells is called hemolysis.

E. STAINING WITH WRIGHT'S STAIN

Stain one slide at a time, and continue trying until you obtain a perfectly-stained slide.

1. Cover the blood smear with Wright's stain, undiluted, counting the drops as you add it.

2. After about one minute, add an amount of distilled water to the slide equal to the amount of stain added. A metallic scum will appear on the surface. After the scum has appeared, let the slide stand for an additional two or three minutes.

3. Rinse the slide in acidulated water (1 drop of acetic acid to 50 ml. of distilled water) and continue rinsing until the film appears to be pinkish-orange when held to the light.

4. Blot gently with paper toweling, and let the slide dry in the air.

5. The slide needs no further treatment, and will keep indefinitely. However, if you wish to protect the smear, cement a coverglass onto the area where the smear fades to nothing. Do this by putting a drop of Canada balsam onto the slide, and then placing a coverglass on top of the balsam.

When finished with the staining, you will have a permanent slide similar to the prepared slides provided in VII-B above, but it will be made with you own blood.

LABORATORY WEEK 14:
GROSS MORPHOLOGY OF VERTEBRATE ORGANS

The bullfrog, Rana catesbeiana, is used as a specimen of a typical vertebrate.

I. MOUTH

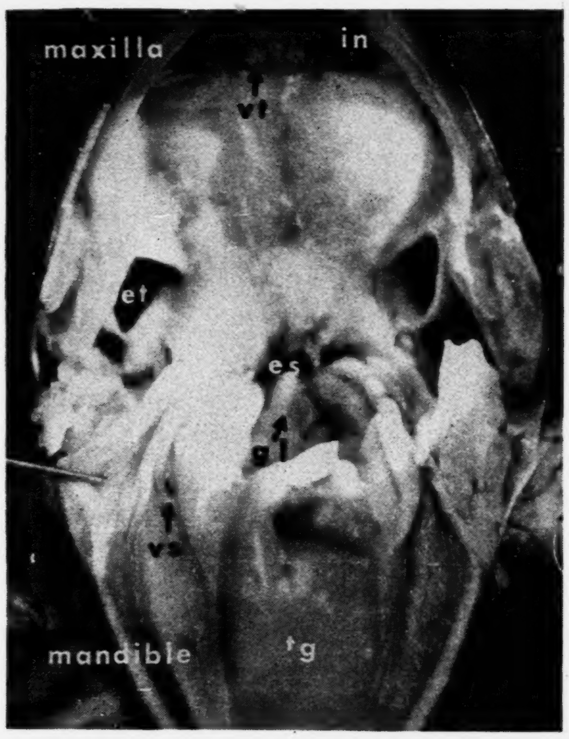
A. DISSECTION DIRECTIONS

Open the mouth to its fullest extent. You will have to pry the jaws apart, but once you have do so it will stay more or less open. Insert your scalpel or scissors into the most posterior angle of the jaw and cut through the bone and flesh (see Fig. 14.1). Do this on each side. This will allow you to get the mouth open wider than before, so you can observe more clearly. If necessary, wash the mouth out under running water.

← Fig. 14.1 Cutting through the angles of the jaw.

Fig. 14.2 →

es = esophagus,
et = eustachian tube,
gl = glottis,
in = internal nares,
tg = tongue,
vs = vocal sac opening,
vt = vomerine teeth.



B. OBSERVATIONS TO BE MADE (see Fig. 14.2)

1. Along the margin of the upper jaw, is a deep groove into which the ridge-like edge of the lower jaw fits. Run your finger or a needle along the edges of the jaws and find the teeth. If on the upper jaw, they are called maxillary teeth; if on the lower jaw they are called mandibular teeth. Which do you find? Examine any teeth you find with a binocular microscope.

2. In the anterior part of the roof of the mouth, near the midline, find two small patches of teeth, the vomerine teeth. Just lateral to these are two small openings, the nostrils or internal nares. Carefully pass a bristle through the external nares and see that it enters the mouth through the internal nares.

3. The greater part of the roof of the mouth is occupied by two rounded prominences caused by the eyes. Press down on one of the eyes and note that it can be made to project considerably into the mouth cavity.

4. Puncture a tympanic membrane and pass a needle through the eustachian tube into the mouth. This tube connects the middle ear with the mouth cavity. Note carefully where the eustachian tube enters the mouth.

5. The floor of the mouth is stiffened by the thin, strong hyoid cartilage which is not visible and should not be dissected at this time. The tongue is attached to this cartilage. Grasp the tongue with your forceps and pull it out of the mouth. What is its shape? Where is it attached? What is the character of its tip?

6. The glottis is a longitudinal slit, on a little eminence, in the posterior part of the floor of the mouth. It opens into the larynx or voice box. Put the tips of your scissors into the glottis and separate its two sides. Can you see the vocal folds? Does the glottis close again when the scissors are removed? Does it have soft or firm margins?

7. In the male frog, the slit-like openings of the vocal sacs may be present on each side of the floor of the mouth just anterior to the angle of the jaw. The sacs are variable in size, and absent in the female. The system is independent of the vocal cords and allows the frog to croak without breathing, even under water.

8. In the most posterior part of the mouth find the esophagus, the tube down which food passes on its way to the stomach.

II. DISSECTING THE DIGESTIVE AND RESPIRATORY SYSTEMS

Both the digestive and respiratory systems are derived from endoderm (the inner layer of the embryo), both are lined with epithelial tissue throughout the entire length, and are similar in other ways.

Make a cut that will separate the small intestine from the large intestine at the junction of the two (see Fig. 14.3). Lift the small intestine without unravelling or uncoiling it and cut the mesenteries that suspend it from the dorsal side of the body (see Fig. 14.4). While lifting, hold the whole group of organs together, and be particularly careful not to destroy the loop formed by the stomach and the first part of the small intestine, where the pancreas is located. Make sure that the following remain in the body: spleen, large intestine, kidneys, urinary bladder, cloaca, testes or ovaries, and oviducts.

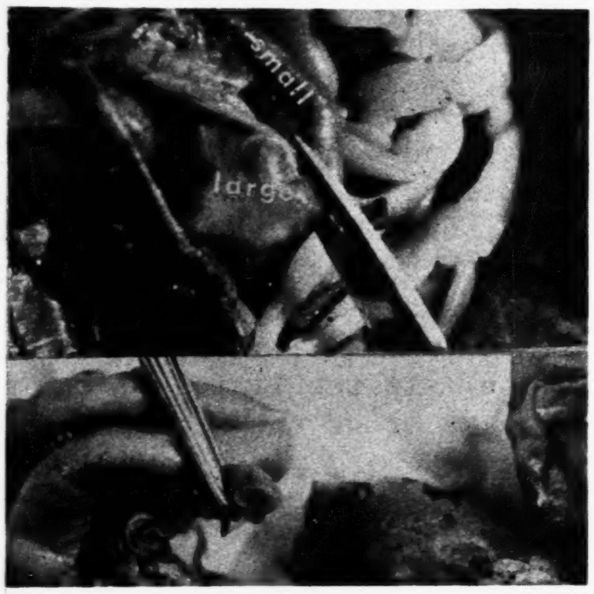


Fig. 14.3 Cutting, separating the small from the large intestine.

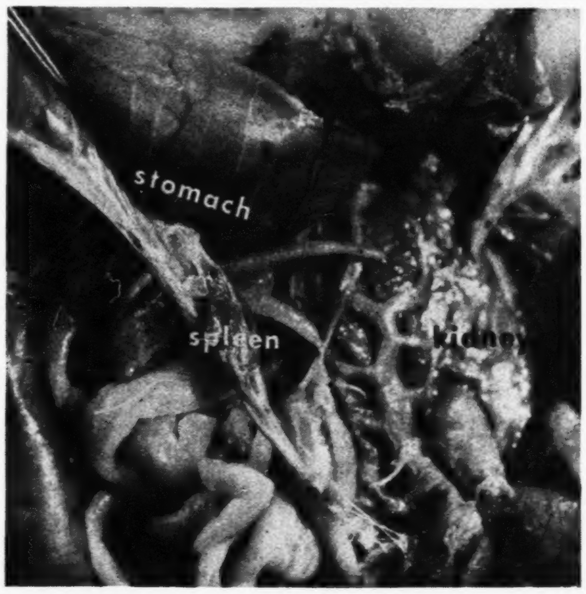


Fig. 14.4 Cutting the mesentery that suspends the stomach.

Trace the small intestine forward to the stomach and then to the esophagus. Cut across the lining of the dorsal surface of the mouth, separating the lower jaw and esophagus from the roof of the mouth (see Fig. 14.5). Cut the systemic arches, carotid arches and other blood vessels as close to the heart as convenient. Now you can lift out all the ventral group of organs: the lower jaw, esophagus, lungs, heart, liver, stomach, pancreas and small intestine.Cut away small blood vessels that prevent the lifting out. Be sure to leave the dorsal aorta and the longer portion of the systemic arches in place.

To dissect the digestive system away from the other organs: Slit the edges of the back of the mouth, and cut transversely across the lower jaw just between the glottis and the esophagus (see Fig. 14.6). Lift up on the glottis and trachea, and cut mesenteries and blood vessels carefully so that you will have the glottis, trachea, lungs and heart in one piece and the esophagus, stomach, liver, etc. in the other piece (see Fig. 14.7). Make the following observations and dissections from the dorsal side of the dissected digestive system:

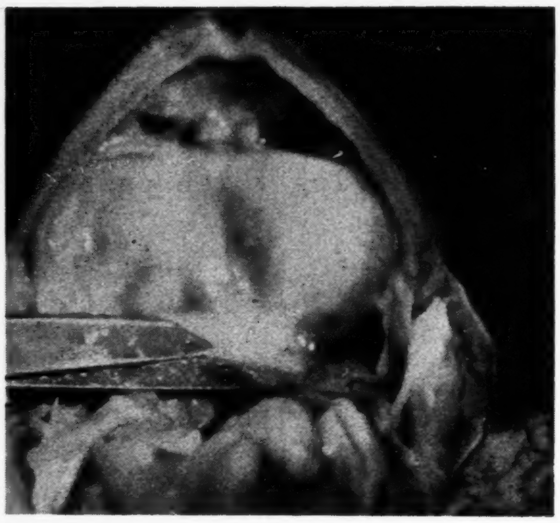


Fig. 14.5 Cutting across roof of mouth. View of upper jaw.

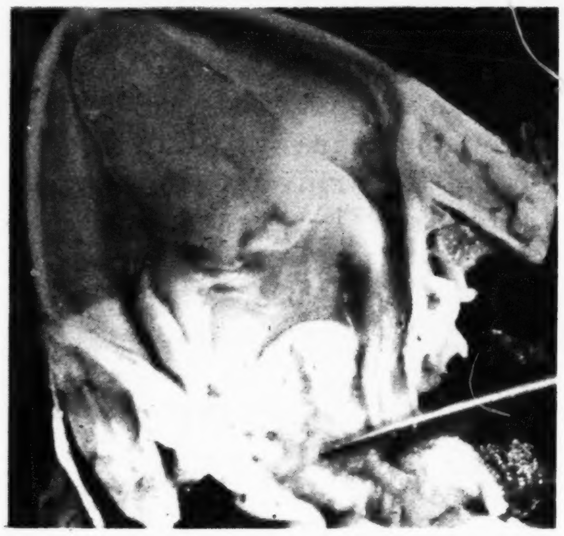


Fig. 14.6 Cutting between glottis and esophagus. View of lower jaw.

A. ESOPHAGUS

Slit open the entire length of the esophagus, and wash the interior with water if necessary. Using a binocular microscope and good light, examine the character of the lining: folds, projections, cilia, etc. Be ready to compare this lining with the lining of the other parts of the digestive system. Trace the esophagus to its posterior ending at the cardiac sphincter, a distinct muscular constriction which, in life, opens and closes allowing food to pass into the stomach. Is the lining of the sphincter more like the lining of the esophagus or of the stomach?

B. STOMACH

Slit open the entire length of the stomach, remove any food in it, and wash the lining with water if necessary . Examine the lining as above Note that it is not uniform, but it varies with the region. Find the three regions of the stomach:(see Fig. 14.8), the upper cardiac region near the cardiac sphincter, the large central fundic region which makes up the greatest part of the stomach, and the lower pyloric region. Under the binocular look for the very small openings of the numerous gastric glands. Is the distribution of these the same in the three regions of the stomach? Find the pyloric sphincter at the lower end of the stomach. This muscle, like the cardiac sphincter, has the ability to open and close, to allow passage of digestive contents into the small intestine.

C. SMALL INTESTINE

Unravel the small intestine, but do not pull apart the loop between the stomach and small intestine, where the pancreas is located (see Fig. 12.5). Measure the length of this intestine, and compare it with the length of the frog's body. Then compare this ratio with the one obtained for the frog larva last term (Lab Week 10). Look up the length of the human intestine, and the length of a human body, and make a ratio of this information also. Can you correlate these facts with the food eaten in each case?

The small intestine is divided into three portions, although you cannot distinguish the three parts from the outside. The difference is mainly in the number and distribution of villi, which are very thin finger-like projections of the lining of the intestine, that give a velvety nap to the lining. Cut open and wash the small intestine throughout its length, and determine the distribution of villi in the following three parts of the small intestine:

1. The duodenum, the first, anterior part, a few centimeters in length.

2. The jejenum, the middle part, variable in length.

3. The ileum, the posterior part, a few centimeters in length.

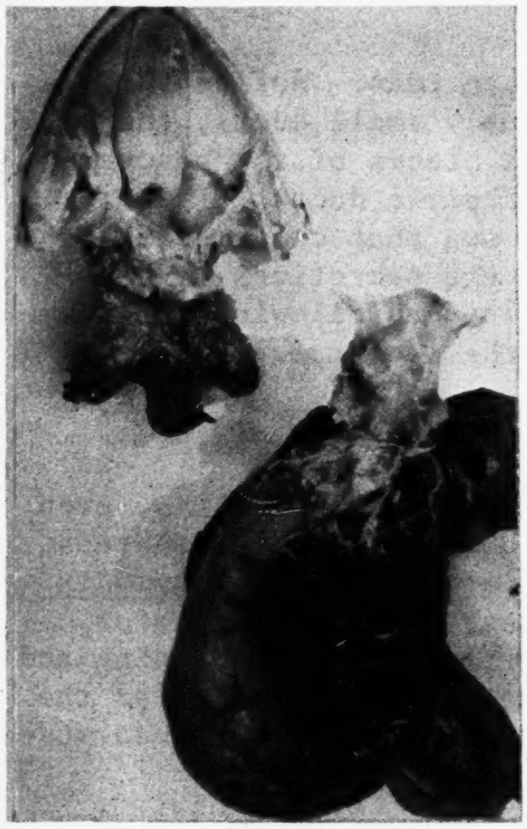


Fig. 14.7 The two pieces resulting from dissection.

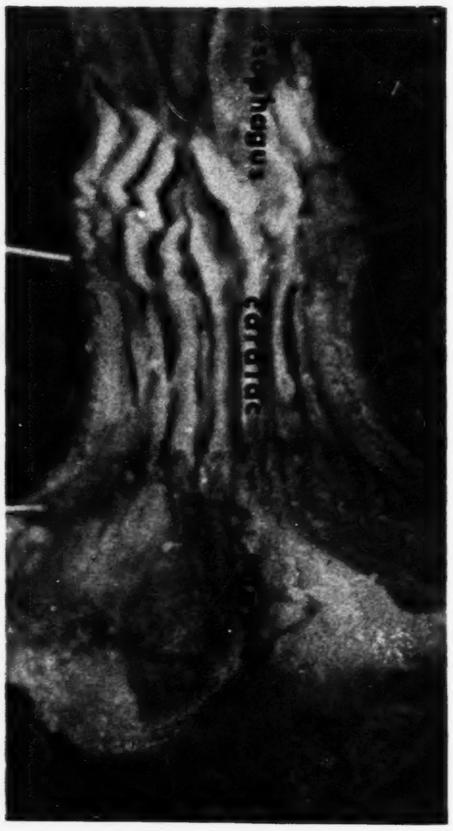


Fig. 14.8 Esophagus and stomach opened. Three parts of stomach shown.

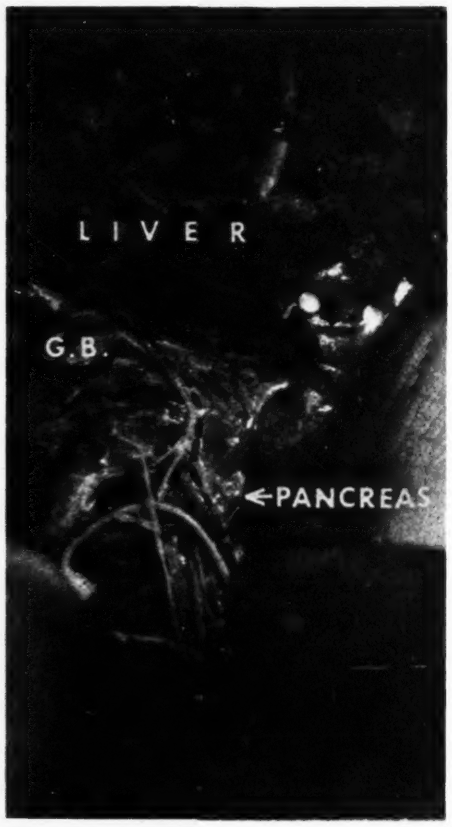


Fig. 14.9 Dissected Liver substance. Route of bile duct is dashed line. One fine hepatic duct shown.

D. LIVER

Examine the various lobes of the liver. How many lobes are there? Which is the largest? The smallest? Which lobe is subdivided? Find the green-colored gall bladder, and be able to describe its location. On the dorsal surface of the central lobes there are several light-colored lines, the hepatic ducts, which collect bile from the tiny lobules of the liver where it is manufactured. Find these ducts, and then find where they all join together, forming the bile duct (see Fig. 14.9). Notice that neither the bile duct nor the hepatic ducts lead either to or from the gall bladder. Instead, there are cystic ducts (one, two or three) that connect the gall bladder to the bile duct. From these facts, what do you conclude about the function of the gall bladder?

Follow the bile duct posteriorly, to its junction with the duodenum. It usually either follows the hepatic portal vein, or passes through the substance of the pancreas, or both.

E. PANCREAS

Follow the bile duct into the substance of the pancreas. Notice that as the duct passes through the gland, it receives many small ducts, the pancreatic ducts. With forceps, pick away small pieces of the pancreas and find the number and distribution of the pancreatic ducts. (Note: in the human, the pancreas has two ducts of its own that carry secretions to the duodenum.) After the bile duct picks up the secretions of the pancreas, it is called the common bile duct. Continue following the common bile duct until you find where it empties into the duodenum.

F. COMPARISON WITH A HUMAN MODEL

The digestive systems of the frog and human are remarkably similar, even in details. The major differences will be in the ducts of the pancreas (described above), in the presence in the human of a vermiform appendix at the junction of the small and large intestines, and in the greater length of the large intestine, with ascending, transverse, descending and sigmoid portions in the human. The human has no cloaca, but instead the digestive system opens directly through the anus. Examine the human model, noting the differences.

G. LARYNX

Remove the heart from its position posterior and ventral to the larynx. Leave the larynx and lungs attached to the lower jaw. The larynx, just posterior to the glottis, is a chamber through which air passes on its way to the lungs. Carefully make a longitudinal slit in the ventral wall of the larynx. Spread the margins of the slit apart and look inside the larynx for the vocal folds, a pair of folds of the wall of the larynx that extend lengthwise of the chamber. Sound is produced by pressing these folds together and forcing air between them. Note that the larynx is held in its shape by the hyoid apparatus, a shield-shaped piece of cartilage, and by rings of cartilage.

H. BRONCHI

Two short tubes that connect the larynx to the lungs. Are these held open by cartilage rings also?

I. LUNGS

Slit the wall of a lung. Note that the inner wall is divided into small chambers, the alveoli. What is the value in this arrangement?

J. COMPARISION WITH A HUMAN MODEL

Note that the human system is expanded, and contains more parts. Find the trachea, which carries air from the larynx to the bronchi, and the bronchioles which carry air from the bronchi to the lungs. Also see that the entire human lung is divided into alveoli, not just the outer wall as in the frog.

III. DISSECTING THE URINARY AND REPRODUCTIVE SYSTEMS

Although their functions are very different, the urinary and reproductive systems are bound together very closely. They develop from the same general region in the embryo, and in the adult they often use the same ducts and external openings. Because of their close structural union, they are conveniently dissected and studied together as the urogenital system.

Find the large intestine, which you previously severed from the small intestine. Trace it posteriorly until it narrows and becomes thicker-walled, and gradually merges into the cloaca (= "sewer"). The cloaca handles all products produced in this area -- feces, urine, eggs and sperm -- and empties them to the outside through the anus. How far may one trace the cloaca without further dissection?

From the ventral surface of the frog, cut off one leg in the region of the acetabulum. Dissect from the cut surface, medially, until you have laid bare the entire cloaca (see Fig. 14.10). Be careful to leave the spinal nerves, dorsal aorta and systemic arches in place in the frog. Lift out the anus and cloaca, together with the urinary bladder, kidneys, ovaries or testes, fat bodies, oviducts, large intestine and urinary ducts. Cut away small blood vessels where necessary. Handle the organs carefully, and place under water for examination.

Note: in addition to dissecting your own frog, you will need to examine a specimen of the opposite sex, so at this point you will need to collaborate with another student.

A. URINARY SYSTEM (see Fig. 14.11) FIND THE FOLLOWING:

1. Blue-colored kidneys (why are they blue?) which remove wastes from the blood. Kidneys are the major organs that maintain the normal constitution of the blood. Find also the adrenal glands, which are light-colored lines that run longitudinally down the mid-ventral side of each kidney. Although these are not urinary structures, they are derived embryonically from tissue that is much like kidneys in structure.

2. Light-colored urinary ducts, extending down the lateral border of each kidney, possibly paralleling the renal portal veins. Many small ducts carry urine from the substance of the kidney into the urinary duct. Follow the urinary ducts to the places where they reach the cloaca, one on each lateral border. Note that they do not lead to the urinary bladder.

3. The thin-walled, sac-like urinary bladder, attached to the ventral side of the cloaca. What route does urine need to follow, to get from the kidneys to the urinary bladder? What do you think might make urine go there?

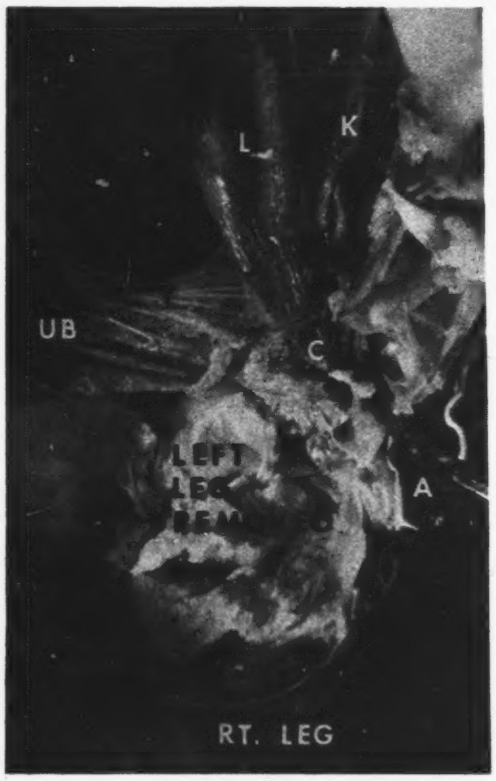


Fig. 14.10 Dissection of cloaca.

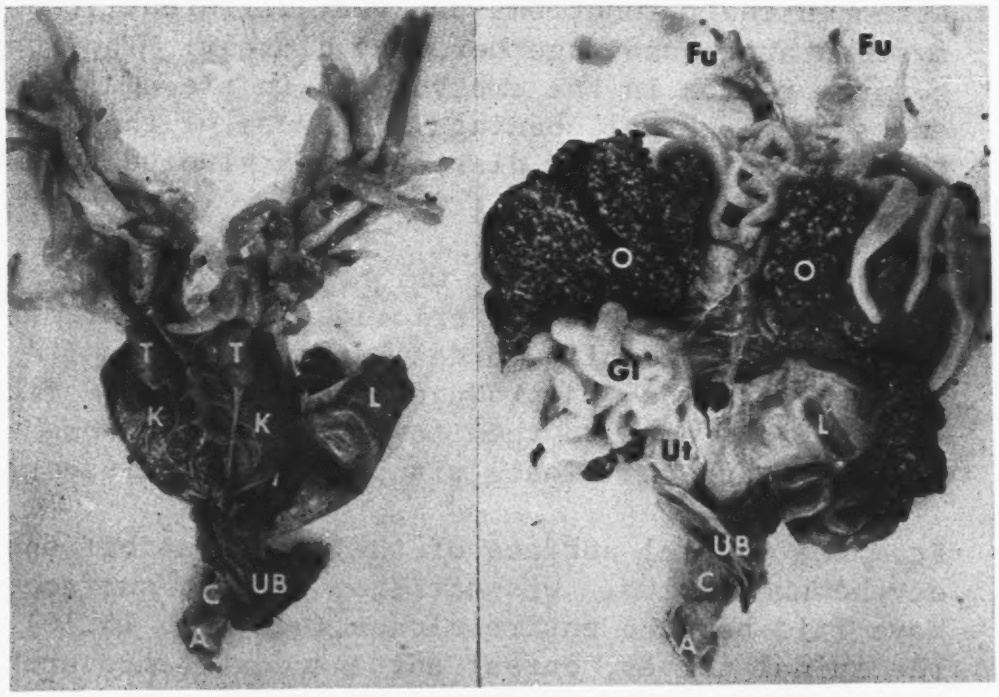


Fig. 14.11 Male and female reproductive systems. A=anus, C=cloaca, K=kidney, L=large intestine, O=ovary, T=testis, UB=urinary bladder. Parts of oviduct shown are Fu=funnel, Gl=glandular portion, Ut=uterine portion.

B. MALE REPRODUCTIVE SYSTEM. FIND THE FOLLOWING:

1. The testes are ovoid, light-colored organs attached one to each kidney. Often they have several finger-shaped yellow fat bodies, attached to their anterior borders.

2. Stretch the mesentery between the testes and kidney, and examine with strong light under the binocular, using a dark background. You should be able to see several tiny light-colored genital ducts lying in the mesentery, extending between the two organs.

3. The genital ducts connect onto small urinary ducts inside the substance of the kidney, and from there on, the sperm follow ducts of the urinary system to the outside. This use of urinary ducts for reproductive function makes the urinary duct become a vas deferens. (This is an example of "duct-stealing", which has occurred continuously in the evolutionary history of vertebrates' reproductive systems. In higher vertebrates, the urinary system abandons this whole series of ducts, originally made by it, and produces a whole new set that connects onto the urinary bladder.) Note below that the female frog has its own set of reproductive ducts, separate from the urinary system.

C. FEMALE REPRODUCTIVE SYSTEM. FIND THE FOLLOWING:

1. The masses of eggs, one on each side of the body, that have been in your way all this time and which you probably wanted to remove, are the two ovaries.

2. The oviducts extend from the armpit region to the cloaca, made of three parts that merge into each other:

    a) The middle glandular portion, comprising the greatest length of the tube. In this part, the jelly-like substance is secreted which will enclose the eggs when they are laid.
    b) The anterior thin-walled portion, extending forward dorsal to the lungs. Trace it forward, and find the funnel at its tip. The eggs enter the oviduct at this point.
    c) The posterior uterine portion. Here the oviduct expands into a wide, membranous, thin-walled storage sac in which the eggs are stored before they are laid (through the cloaca and anus).

D. COMPARISON WITH A HUMAN MODEL

1. Urinary system. Note the differences:

    a) The human has developed both a new kind of kidney and new external attachments for it. The kidneys occupy the same relative position in the human as in the frog, but much different shape.
    b) The ureters (urinary ducts) leave the kidneys medially rather than laterally, and carry urine directly to the urinary bladder rather than to a cloaca.
    c) Another duct, the urethra, carries urine from the urinary bladder to the outside of the body. In both sexes, urine does not leave the body through the anus, but through a separate opening at the end of the urethra.

2. Reproductive system, female. Notice that the model may be made either male or female by interchanging reproductive systems. Note these differences from the female frog:

    a) Great reduction in size of the ovaries, made possible both by reduction in size of the ova and by reduction in number of ova. The ovaries therefore can occupy a position low in the abdomen, and the oviducts can be quite short. (Oviducts in the human are sometimes called "fallopian tubes".)
    b) The two oviducts fuse together to form a large uterus, which is used in sheltering and nurturing the developing embryo. The uterus connects to the outside through the vagina.

3. Reproductive system, male. Note these differences from the male frog:

   a) Sperm in the human need not pass through the kidneys to get to the ureters -- the reproductive system has taken over that system of tubes entirely, and the number of sperm ducts has been reduced to one on each side, called the ductus deferens.

   b) The ductus deferens from each side fuses with the new urethra just below the bladder, and the urethra acts as a sperm-carrying duct as well as a urinary structure.

   c) In the mature human male the system has become further complicated due to the fact that the testes migrate from their original position in the body cavity to an abnormal position in the scrotum. As they migrate, they pull with them the tubes that connect them with the urethra. This explains the peculiar fact that sperm must travel up through the body cavity before they leave the body.

IV. DISSECTING THE NERVOUS SYSTEM

A. THE NERVOUS SYSTEM OF VERTEBRATES IS DIVIDED INTO THREE MAJOR PARTS, NAMELY:

1. The central nervous system, mainly concerned with coordination of stimuli and responses. Included here are the brain and spinal cord.

2. The peripheral nervous system, concerned with transporting of stimuli from one part of the body to another. There are two types of nerves in this system:

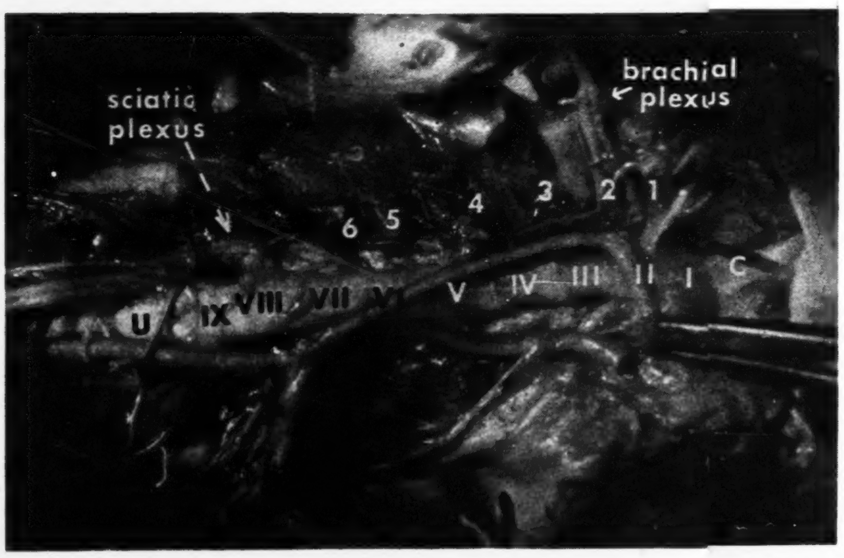
   a) Cranial nerves, arising from the brain.
   b) Spinal nerves, arising from the spinal cord.

3. The autonomic nervous system, which controls the involuntary activities of the body: those of which you are unaware and over which you have no direct control -- e.g., digestive action.

B. THE SPINAL NERVES

At this time there is very little left of your bullfrog, so from the ventral side you can identify the vertebrae and the urostyle. The spinal nerves are white threads lying against the dorsal body wall on each side of the vertebral column. Trace the nerves laterally. The most conspicuous ones are the second, leading to the arm, and the group made up of the seventh, eight and ninth which lead to the leg (see Fig. 14.1

Fig. 14.12
Dissection showing vertebrae and spinal nerves.
I-IX are vertebrae; arabic numbers are nerves.
U=urostyle, C=cranium.



1. Ten pairs of spinal nerves arise from the sides of the spinal cord. The point of exit is through the space between adjacent vertebrae, and this point is covered by a white mass, the calcareous body. The first spinal nerve leaves the spinal cord in the space between the first and second vertebrae; the second between the second and third vertebrae, etc. The tenth spinal nerve leaves the spinal cord through a hole in the urostyle, and is very small. (Note: in keeping laboratory data, records are clearer if you give Roman numerals to vertebrae and Arabic numbers to nerves)

2. The plexuses

    a) The brachial plexus is made up of all of the second spinal nerve plus parts of the first and third. Note that not all of each nerve joins in making the plexus: the first usually contributes only on small branch; its main branch goes to the muscles of the shoulder and back. The second spinal nerve is the largest and most conspicuous of the three that form the plexus -- a large white cord lying at right angles to the spinal column.

    b) The sciatic plexus is made up of parts of the seventh, eight, ninth and (usually) tenth spinal nerves. Posterior to the plexus, several nerves are formed which proceed to the hind quarters of the body. Of these, the largest is the sciatic nerve. Trace this nerve to the large muscle of the leg.

C. THE AUTONOMIC NERVOUS SYSTEM

Lift the dorsal aorta slightly and notice the two slender longitudinal nerve trunks attached to its dorsal side, one on each side of the vertebral column Each trunk has ten enlargements on it, and from these enlargements (ganglia) there are connecting nerves to the ten spinal nerves. Other nerves, arising from these ganglia, go to the digestive tract, the heart, and other organs; and help to control the involuntary activities of the body. Since you have already removed these organs you cannot see the connections.

Nearly all the autonomic system that you see here, belongs to the thoraco-lumbar system, so called because it is attached to the central nervous system in the thorax and lumbar regions. The other autonomic system, the cranio-sacral system, is connected to the central nervous system in the cranium (brain) and the sacral regions; connected to nerves nine and ten. You will not be able to see very much of the cranio-sacral system.

D. THE BRAIN AND SPINAL CORD

Remove the skin and muscles in a narrow strip from the middle of the top of the head and trunk. This exposes the cranium and the neural spines of the vertebrae (see Fig. 14.13). By bending the head slightly downward, a space appears between the posterior end of the cranium and the first vertebra. This space has been previously identified as the foramen magnum. Insert one blade of a fine pair of scissors into the skull through the opening, keeping the point well up against the bone and away from the brain. Make a cut along the side of the cranium, as far forward as the eyes. Make a similar cut on the other side, and lift off the roof of the cranium with forceps. The brain will now be exposed (see Fig. 14.14). Similarly cut posteriorly through the dorsal sides of the neural canal and expose the spinal cord. Observe as much as you can without further dissection; then remove the brain and spinal cord by cutting the nerves. When the brain is removed it is best observed under water.

1. The brain and cranial nerves. (Note: there are ten pairs of cranial nerves, only two of which are mentioned here.) The brain is covered with a delicate, dark membrane called the pia mater. Carefully remove this, and observe. The brain is made up of six parts. From anterior to posterior they are:

    a) The olfactory lobes, one on each side. These make up the most anterior part of the brain. They are united in the midline. Each gives off anteriorly an olfactory nerve (the first pair of cranial nerves) and is separated from the cerebral hemispheres behind them by a slight constriction.

    b) The cerebral hemispheres, one on each side just posterior to the olfactory lobes.

    c) The diencephalon, a small depressed region just posterior to the cerebral hemispheres. The dorsal side is the place where the pineal body was located, but this structure is usually torn off in dissection. The optic nerves arise from the ventral surface of the diencephalon and may be seen by gently lifting and looking underneath. Each optic nerve penetrates the adjacent eyeball.

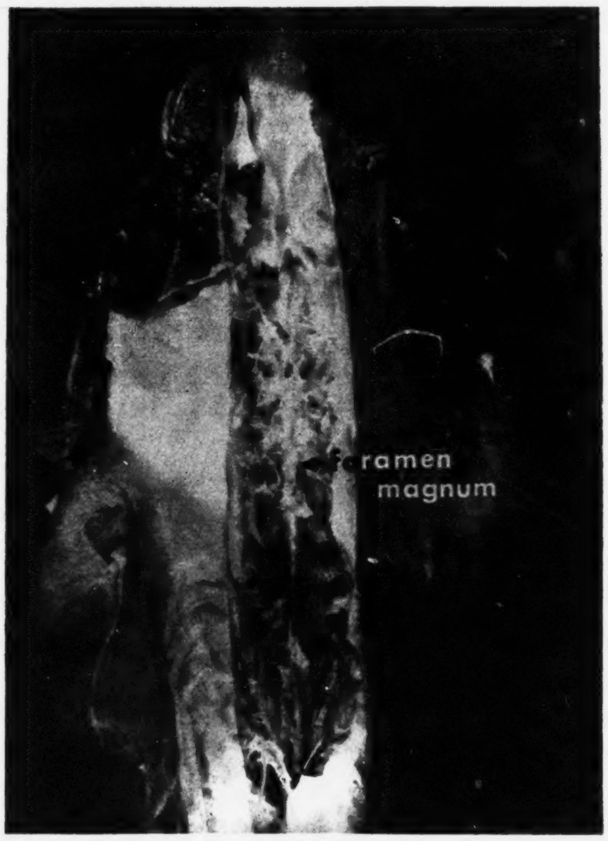


Fig. 14.13 Skin and muscles removed, cranium and neural spines exposed.

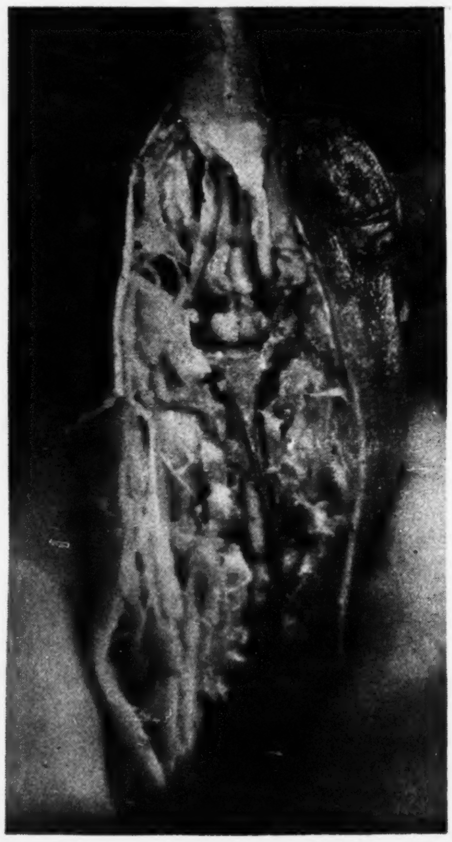


Fig. 14.14 Brain and spinal cord exposed after removal of cranium and dorsal sides of vertebrae.

d) The optic lobes are two rounded bodies, just posterior to the diencephalon.

e) The cerebellum is a narrow transverse ridge just posterior to the optic lobes, separated from them by a deep groove.

f) The medulla oblongata, directly posterior to the cerebellum forms the lateral walls and floor of the triangular depression known as the fourth ventricle of the brain. The anterior boundary of the fourth ventricle is the cerebellum. The fourth ventricle is covered by a dark-colored vascular (= filled with blood vessels) membrane. Posteriorly, the medulla oblongata is continuous with the spinal cord.

2. The spinal cord is continuous with the brain, and ends in a terminal filament in the urostyle. Notice that there are swellings at the points in the cord where the brachial and sciatic plexus nerves branch off.

3. Dissection directions: Remove the brain and spinal cord by cutting the nerves attached to them. Place the brain at once in a dish of water, which will support the tissues and prevent their collapsing (see Fig. 14.15).

4. On the ventral side of the brain observe the following:

   a) The optic chiasma is cross-shaped structure made up of the two optic nerves, and is located on the ventral side of the diencephalon.
   b) The infundibulum, a two-lobed stalk on the ventral side of the brain, posterior to the optic chiasma. In life, the pituitary gland is attached to the infundibulum, but it probably came off in removing the brain. Try to find the pituitary gland inside the brain case, if it is not attached to the infundibulum.

5. Dissection directions: Slice off the dorsal surface of the brain with a sharp scalpel.

6. Inside the brain, observe the cavities or ventricles of the brain. The first and second are inside the cerebral hemispheres, the third is in the diencephalon, and the optic ventricles are in the optic lobes. These ventricles are all continuous with each other and with the fourth ventricle described above, as well as with the central canal of the spinal cord.

7. Compare the brain of the frog with the model of the human brain. Notice the comparative sizes of the various parts, and the curvature in the human brain.

V. DISSECTING THE EAR (see Fig. 14.16)

Cut the skin around the tympanic membrane, and carefully remove it. Find the cavity thus exposed, the middle ear cavity. Within this cavity is the columella, a rod of bone and cartilage. One end of the columella is attached to the tympanic membrane, while the other end of it enters the auditory capsule. The columella serves to transmit vibrations from the tympanic membrane to the inner ear, which lies within the auditory capsule. The middle ear cavity communicates with the mouth cavity through the eustachian tube.

Examine a model of the human ear, and find out how the ear works. Note that instead of a single columella, there are three bones in the human middle ear: the hammer, anvil and stirrup. Notice also the semicircular canals, used in maintaining equilibrium. What are the two functions of the middle ear?

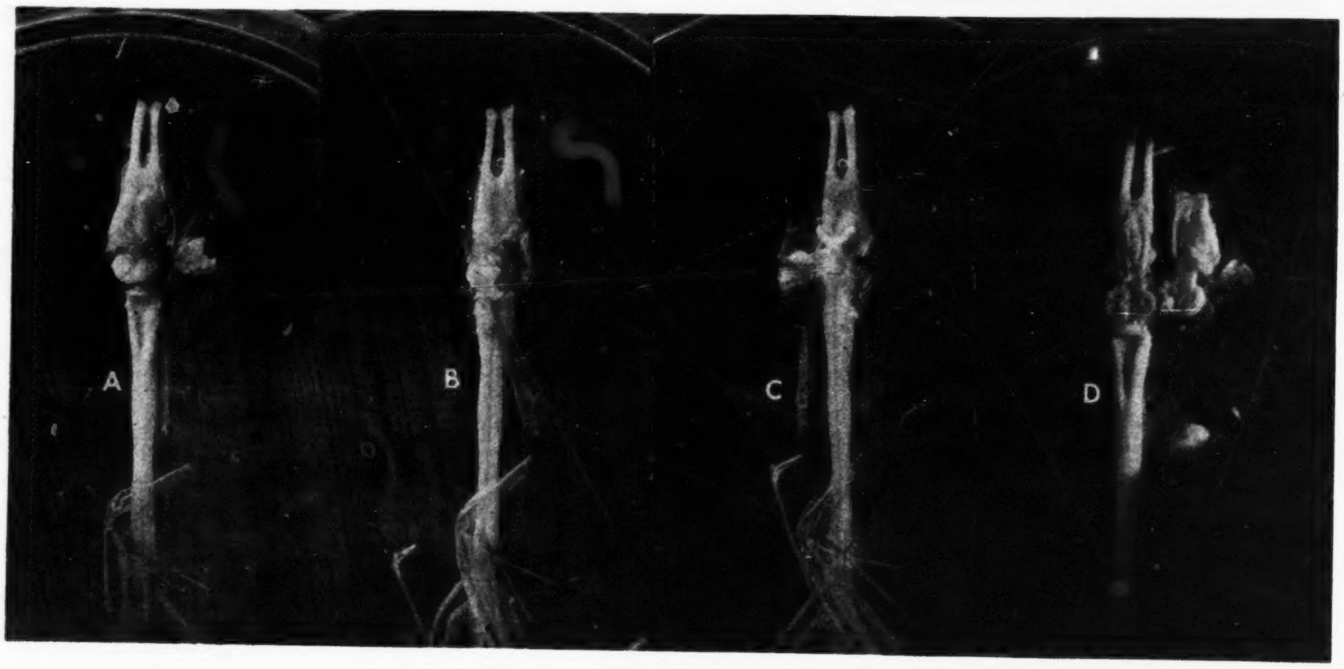


Fig. 14.15 Frog brain and spinal cord dissected out.

A: dorsal view, misplaced pituitary gland to right of brain.

B: ventral view, pituitary gland still attached to infundibulum.

C: ventral view, pituitary gland pulled to left.

D: dorsal view, dorsal side of brain sliced off to show ventricles inside.

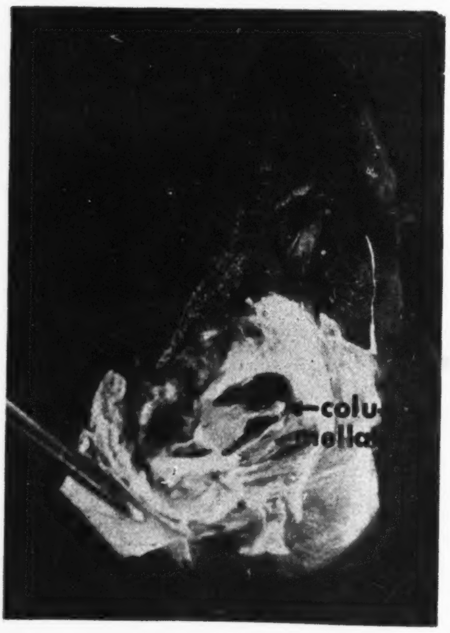


Fig. 14.16
Dissecting the ear.

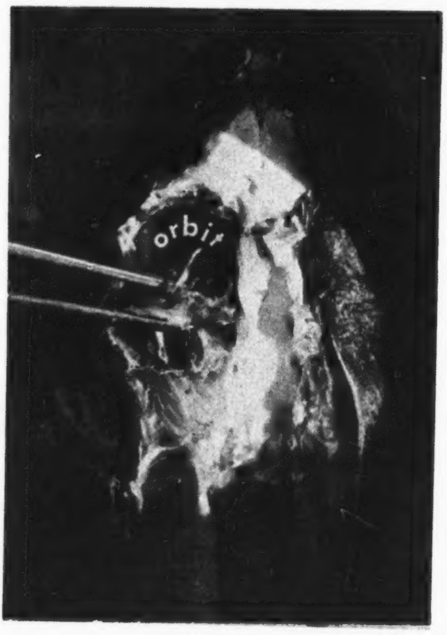


Fig. 14.17
Dissecting the eye.

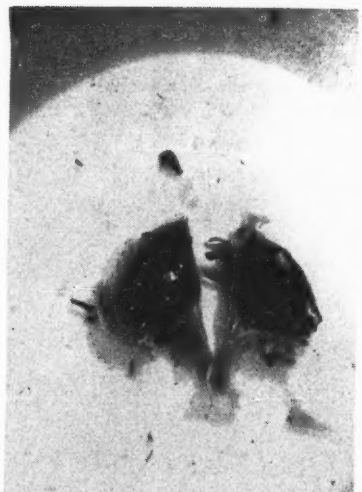


Fig. 14.18
Interior of the eye exposed.

VI. DISSECTING THE EYE (see Fig. 14.17)

Expose the eye by cutting with a sharp scalpel around it. Notice that it is attached to the orbit both by a heavy layer of connective tissue and by muscles that move it. Remove the eyeball entirely by cutting the connective tissue and muscles. The eyeball is covered all over the outside by a very tough sclerotic coat. This coat is continuous over the front of the eye as the cornea, but this is transparent and not as tough as the rest of the sclerotic coat. Slit the eyeball by a cut passing through the middle of the cornea. Remove the firm, solid, transparent lens, which is loose inside the eyeball. (The lens is not loose in a living frog.) Extend the cut so as to expose the interior of the eye. (see Fig. 14.18)

The space between the lens and cornea contains, in life, a watery fluid, the aqueous humor. The space behind the lens was filled by a gelatinous vitreous body. The chorioid coat is the black layer lining the sclerotic and is continuous in the front of the eye with the colored iris. The retina is the delicate transparent inner lining of the eyeball, where the sensitive nerve cells are located. The retina is readily detached from the chorioid except where the optic nerve enters the retina.

LABORATORY WEEK 15:
MICRO-MORPHOLOGY OF EPITHELIUM AND ITS ASSOCIATED ORGANS

I. BACKGROUND

Epithelial tissues are those that limit surfaces, and are derived embryologically from both ectoderm and endoderm. Those derived from ectoderm cover the outside of the body and line the external glands; those derived from endoderm line the internal organs and the internal glands. The cells of epithelial tissues lie close together, with little space between them for matrix such as you found in connective tissue. The cells are found in flat layers or sheets, often only a single cell-layer thick and never more than a few cell layers thick. Epithelial cells may be classified by their shape (squamous, cuboidal or columnar), by their attachments (ciliated, flagellated), or by their function (protective, glandular, nervous).

When slides of this tissue are prepared, they may be sectioned so that the cells are in their normal position, or they may be smeared on the slide. In any case, the application of stain to the tissue changes its normal appearance considerably.

In the following study of epithelial tissues, you will first observe one or more types of tissue, and then examine sections of organs in which that type occurs. For example, II and III take up squamous and cuboidal tissues; IV and V then describe how these tissues appear in skin and in a large gland.

II. SQUAMOUS EPITHELIUM FROM FROG SKIN

Squamous cells are flat, found where epithelium is in contact with the air. Where it is stratified (occuring in layers) the outer layers are usually dead and cornified (hardened).

Slide # 40 contains a thin piece of frog skin, one cell-layer thick, mounted flat and whole, and stained. Cells appear large, but remember that they are flattened. Examine the tissue, first with low power and then with high power (see Fig. 15.1). Note the cells, each with a nucleus and cytoplasm. Also note that the cell membranes of adjacent cells are so closely-pressed together, that you cannot distinguish the end of one cell and the beginning of another.

Fig. 15.1 Squamous epithelium, high power

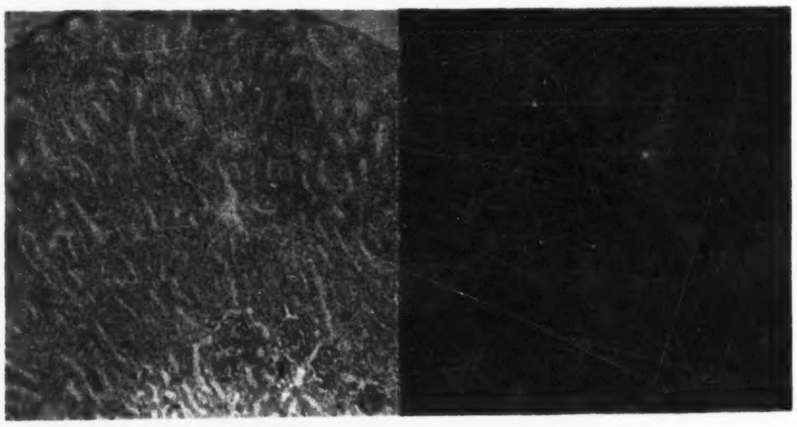


Fig. 15.2 Kidney, cuboidal epithelium,
low power high power.

III. CUBOIDAL EPITHELIUM

Slide #41 is a section of kidney, an organ that is made up almost entirely of tubules. The tubules are lined with cuboidal epithelial cells (see Fig. 15.2). Cuboidal cells are about as high as they are wide, and in section they look square. Cuboidal epithelium is not found in contact with air, but it lines many glands and may be found covering the outside of the body of aquatic animals. Examine the cells lining the uriniferious tubules in the slide, noticing especially the shape of the cells and location of the nucleus in them.

IV. THE SKIN

This organ is made up of a thin outer layer of stratified epithelium called the epidermis, and a thicker layer of interlaced connective tissue known as the dermis. You will study the skin in vertical section -- that is, in a section cut at right angles to the surface.

A. SKIN OF A FROG, SLIDE #42 (see Fig. 15.3)

Find a typical section of the slide, and under low power identify the epidermis and the dermis. Remain on low power except when you want to see details of the cells.

1. The epidermis is made up of stratified epithelium. This means that the epithelium is several cell-layers thick. Its outermost cells are stratified and cornified; a few cells inward from these cells are living squamous epithelial cells. What shape are the innermost cells of the epidermis? These cells make up the germinative layer, which divide rapidly and produce all the outer layers.

2. The dermis is composed of connective tissue with numerous blood vessels running through it. There is a layer of pigment cells (dark, irregular cells) just beneath the epidermis, and often another layer of them at the base of the dermis.

3. The glands are composed of epithelium, but are found in the dermis. They appear as numerous globular spaces, which open to the surface of the epidermis through slender ducts. You will see sections through many glands, but only through a few ducts. A little thought will explain why this is true. Find two kinds of glands:

   a) Mucus glands, in which the lumen appears empty.
   b) Serous glands, in which the lumen appears filled with granules.

   Examine these glands under high power and determine the shape of the cells that line both the lumen and the duct. Are they squamous or cuboidal? Simple or stratified? The mucus glands secrete the slimy fluid with which the frog is covered; the serous glands secrete a fluid which is supposed to be either poisonous or bad-tasting or both.

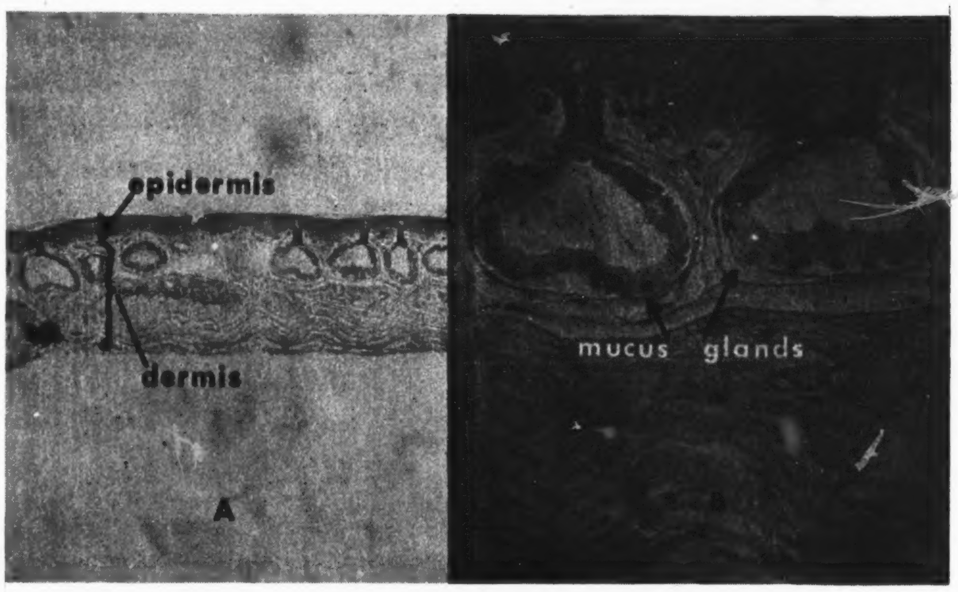

epidermis
dermis
A
mucus glands
B

Fig. 15.3
Frog skin, vertical section.
A low power, B high power.
No serous glands are shown.

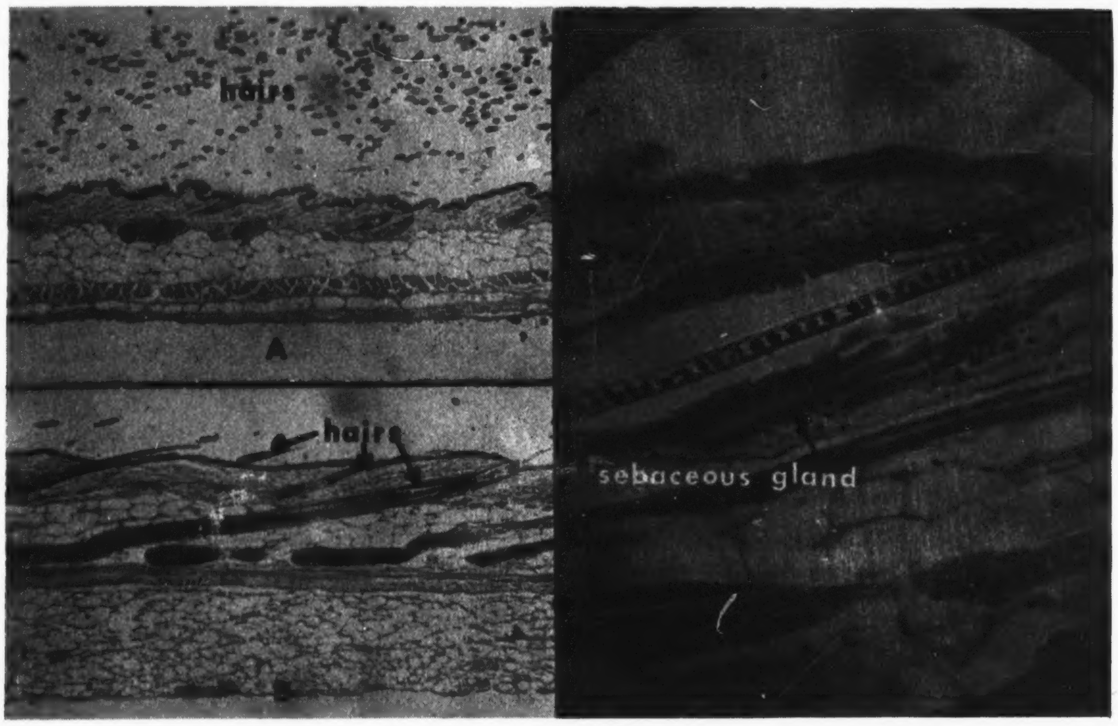
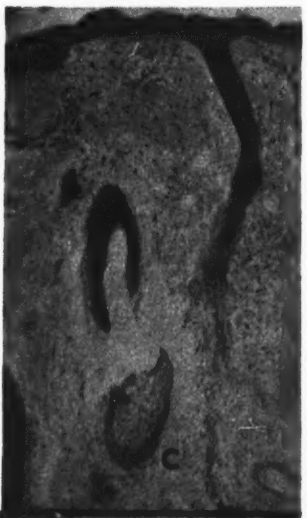

hairs
A
hairs
B
sebaceous gland

Fig. 15.4 Mammal skin, vertical sections. A and B are low power, C is high power. A is cut perpendicular to hairs, B is cut parallel to hairs.



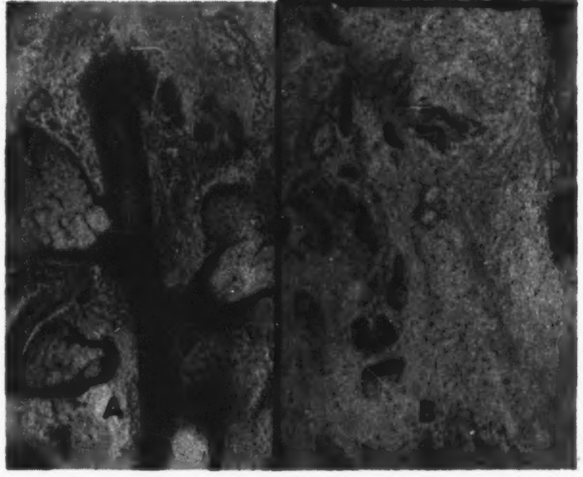


Fig. 15.5 Human skin, vertical section, all views are low power. A shows sebaceous glands around a hair follicle, B is cut through the coiled base of a sweat gland, C shows the duct of a sweat gland.

B. SKIN OF A MAMMAL, SLIDE #43 (see Fig. 15.4)

This skin differs from the skin of the frog, primarily, in the following ways:

1. Epidermis:

   a) Comparatively thicker, with a much heavier and thicker cornified layer, corresponding to the environment of dry air surrounding the animal.
   b) The germinative layer is not flat, but thrown into finger-like projections called papillae. This makes the epidermis appear wavy.

2. Dermis:

   a) Hairs are present. Notice that the follicle of a hair is lined with epithelium. What kind? A large papilla is found at the base of the hair. Find its germinative layer. Notice that the hair is made up of nothing but layers of dead, cornified epithelium.
   b) There are no mucus or serous glands.
   c) Although sweat glands may be found in some mammalian skin, there are none in these slides. A demonstration is provided of a section through human skin, showing sweat glands. They are complex, very coiled, deep in the dermis, with slender ducts leading to the surface. Look for knots of epithelial cells deep in the dermis (see Fig. 15.5).

V. THE LIVER

This large digestive gland owes its epithelial character to the fact that during embryonic life it developed as an outgrowth of the small intestine, which is lined with epithelial tissue. Examine slide #44 of pig liver (see Fig. 15.6).

A. Under low power, see that the organ is divided by connective tissue membranes into many small sections called lobules. Each lobule of the liver is a mixture of circulatory and epithelial elements:

1. At the center of each lobule is a central vein, which is a branch of the hepatic vein (carrying blood from the liver to the heart).

2. The liver cells are modified cuboidal epithelial cells, arranged more-or-less regularly in plates radiating from the central vein. In cross-sections of the lobules, these plates appear to be slender columns of cells, radiating like spokes.

3. The interlobular veins lie in the connective tissue membranes between the lobules. They are branches of the hepatic portal vein, which brings blood from the digestive system to the liver.

Blood circulates slowly from the interlobular vein through the open liver sinusoids (the spaces between the plates, which in these slides are reduced in size due to the drainage of blood from the liver during slide preparation). From the liver sinusoids the blood is collected by the central vein. The blood thus comes into intimate contact with a great many liver cells that line the sinusoids. Recalling the source of the blood in the hepatic portal vein, of what advantage do you think this arrangement is to the animal?

B. Examine a few of the liver cells under high power. How can you determine that they are actually epithelium? Note their similarity to the cuboidal glandular epithelium cells of the frog's mucus glands. Bile, a secretion of these cells, is collected by tiny bile capillaries -- extremely thin-walled ducts that penetrate throughout the lobule. You will find only suggestions that these are present. These bile capillaries join together and form interlobular hepatic ducts, that carry bile to the bile duct and thence to the small intestine.

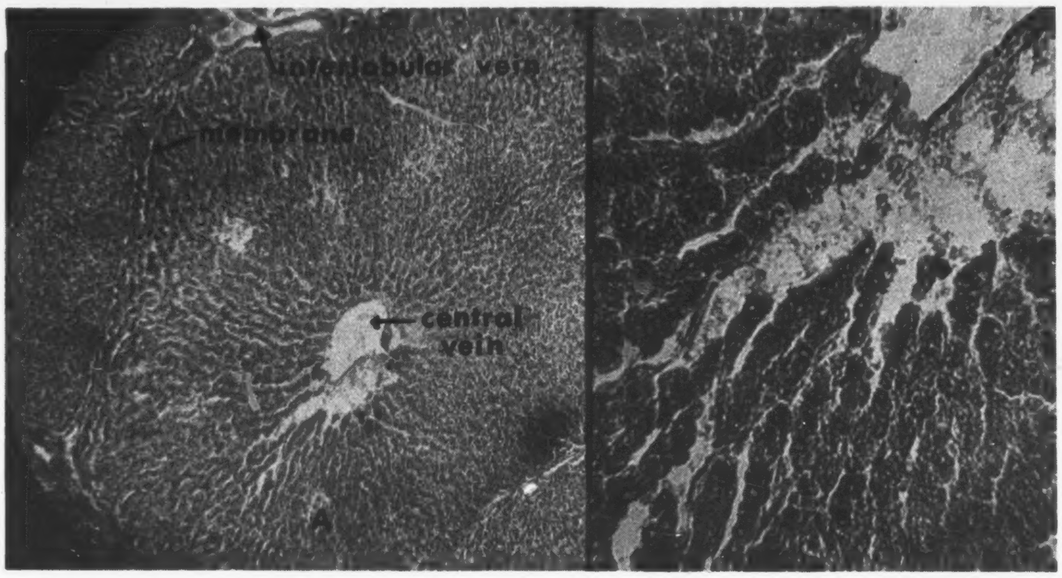


Fig. 15.6
Pig Liver. A is low power, B is high power. A shows nearly all of one lobule. B shows plates of cells radiating around the central vein.

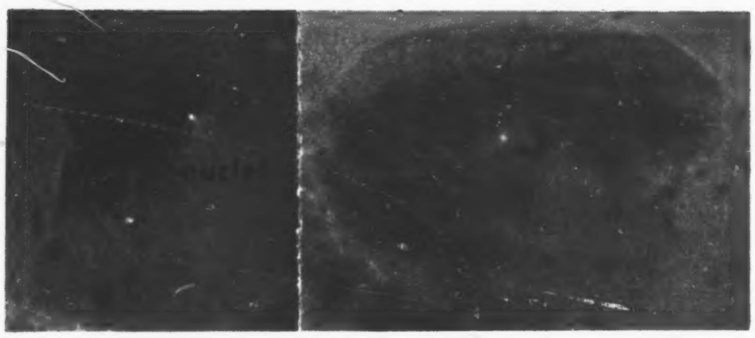


Fig. 15.7 Two high power views of macerated columnar epithelial cells.

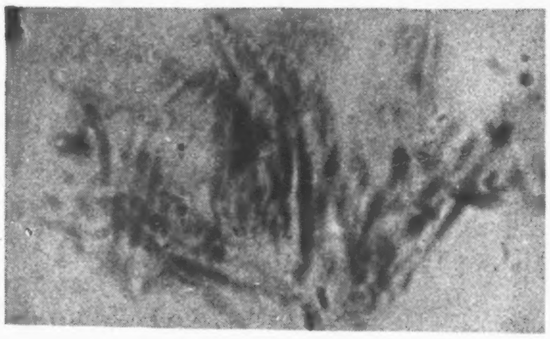


Fig. 15.8 High power view of ciliated columnar epithelial cells.

VI. COLUMNAR EPITHELIUM

Slide #45 (see Fig. 15.7) The tissue on the slide was first soaked in a solution that loosened the cells from each other; and these cells were spread on the slide and stained. They will therefore not be in their normal relationship to each other. Find the cells under low power, then switch to high power to observe their details. They are longer than they are wide, and that the nucleus is situated at one end of the cell. These cells are found in their normal relationship to each other in VII - A and - B below (see Fig. 15.10 and 15.11).

Slide #46. (see Fig. 15.8) This tissue was treated as above. Cells are columr with tiny cilia attached to the free end of the cell opposite the nucleus. In life, the cilia beat, sweeping the surface of the tissue. A demonstration slide is provided of a cross section through a trachea, showing the normal arrangement of these cells (see Fig. 15.9).

VII. THE DIGESTIVE SYSTEM

Essentially, the lining of the digestive system is like the skin: first, in contact with the environment (food) is a layer of epithelium; in this case a single layer of columnar cells. Then, under the epithelium is a layer of connective tissue, containing glands, blood vessels, etc. Added to this are several layers of muscle.

A. STOMACH OF A FROG

Slide #47 is a cross-section through a frog stomach (see Fig. 15.10). Beginning at the lumen, or empty space in the center, and working outwards in the section, identify:

1. The mucosa. This is made of epithelium, connective tissue and muscle. Notice that it includes more than the epidermis of the skin.
    - a) Columnar epithelial cells. Though it appears to be made up of many layers of cells, it is actually only one layer, very much folded. Are the cells ciliated? Notice the position of the nuclei of the cells. You should not confuse the folds of the stomach wall with the gastric glands, which are cylindrical pits embedded vertically in the wall of the stomach. The glands are also lined with a single layer of columnar epithelium, and they produce digestive enzymes which are discharged through the open mouths of the glands into the lumen of the stomach.
    - b) Loose Connective Tissue. Like excelsior stuffing, this fills up the spaces between gastric glands, folds, etc., and between these and the next layer.
    - c) Muscularis mucosae. This is a very thin but definite layer of smooth muscle cells, and it marks the outer edge of the mucosa. High power examination will show that it is made of three layers.

2. The submucosa. This is a layer of dense connective tissue that looks very much like the dermis of the skin. It contains blood vessels, lymph vessels, nerves, etc. It extends into the folds of the stomach, but does not go between the gastric glands.

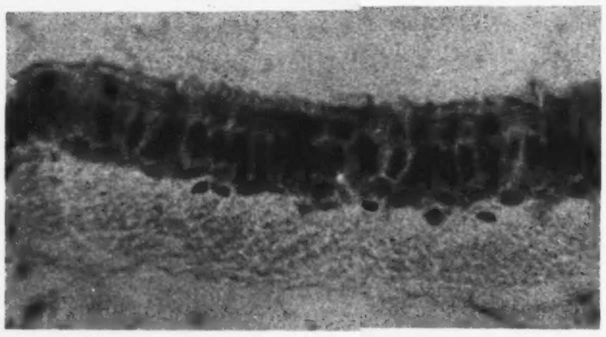


Fig. 15.9
Ciliated columnar
epithelium, high power.
From cross-section of
trachea.

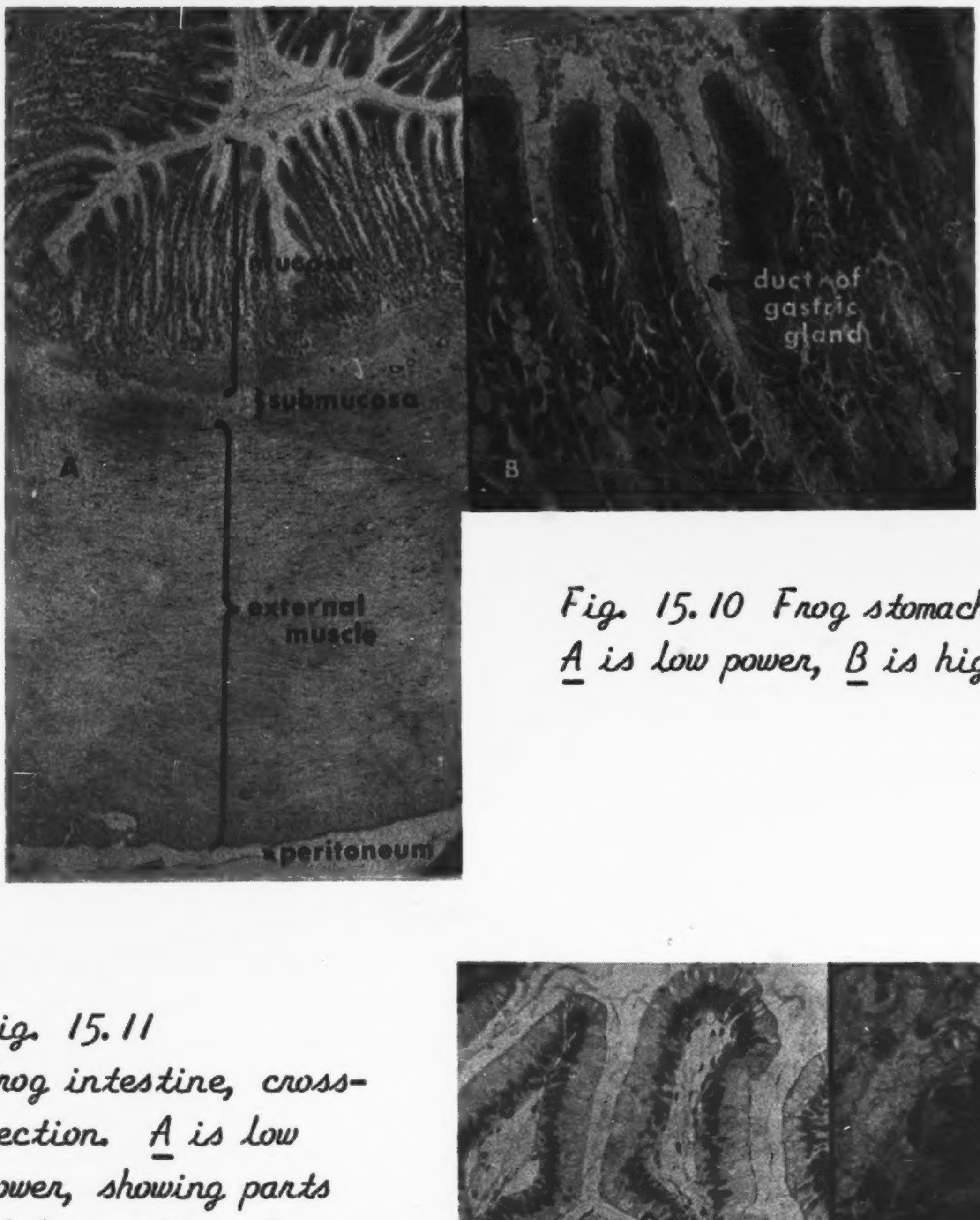
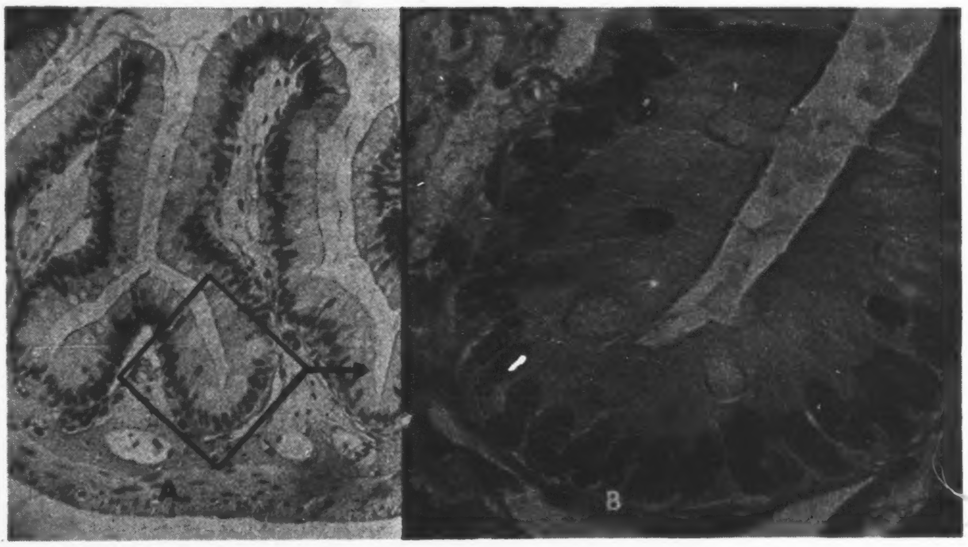

mucosa
submucosa
A
external muscle
peritoneum
duct of gastric gland
B

Fig. 15.10 Frog stomach, cross-section. A is low power, B is high power.

Fig. 15.11
Frog intestine, cross-section. A is low power, showing parts of four villi. B is high power of epithelium including goblet cells.



3. The external muscle. A very thick layer of smooth muscle cells bound together with connective tissue. Each of the three sub-layers encircles the stomach in a spiral:

    a) The circular muscle layer is the thickest, and lies just outside the submucosa. It is in a very tight spiral, so it appears that it is circular around the stomach.
    b) The oblique muscle layer is of variable thickness in different parts of the stomach. The spiral is not as tight as in the circular muscle, so its cells look oblique.
    c) The Longitudinal muscle layer is much thinner than the circular, and is the outermost of the three muscle layers. The spiral is so long that the layer looks longitudinal.

4. The peritoneum is a very thin layer of squamous cells on the very outside of the section. Look carefully under high power.

B. INDEPENDENT STUDY OF THE SMALL INTESTINE OF A FROG. SLIDE # 48 (see Fig. 15.11).

No special instructions are given here for the study of this slide -- this is an independent piece of work. Compare it carefully with the section of the stomach, noting similarities and differences. Your text will contain some information about the special features of this organ.

VIII. NERVOUS TISSUE

A highly-specialized tissue, derived from embryonic epithelium but with very different appearance. Two types of cells make it up: neurons which are large and irregular in shape, and neuroglia cells which support the neurons.

A. ISOLATED CELLS

Slide # 49 is a smear of the ventral part of the spinal cord of an ox (see Fig. 15.12). It shows complete cells. Find a neuron, and examine it under high power. The central part, the cell body, contains the nucleus, and there are many long projections out from the cell body. The largest of these projections is the axon, which arises from a prominent axon hillock. The axon always carries impulses away from the cell body. The other projections are the dendrites, which carry impulses toward the cell body. Small cells present are neuroglia.

B. CROSS SECTION OF THE SPINAL CORD OF A FROG

Study slide # 50 with a binocular microscope. The spinal cord is bilaterally symmetrical, right and left sides are (or should be) mirror images of each other. The ventral surface is flattened with a blood vessel on it; the dorsal surface is rounded. Find:

1. Central cavity, very thin.
2. Dorsal fissure, should divide the dorsal surface into two halves, and extend nearly to the central cavity.
3. Ventral fissure, usually wider and shorter than the dorsal fissure.
4. Gray matter, H-shaped, at the center of the cord. You should see neurons in this, but none in the white matter. Find nerve fibers, neuroglia.
5. White matter, surrounding the gray matter. Nerve fibers, neuroglia.
6. Meninges, or coverings. From inside to outside they are very thin pia mater, the webby-looking arachnoid mater, and the thick dura mater. Many portions of arachnoid and dura may have been lost in preparation.

Examine the slide with the compound microscope, examining the above structures more closely than you can with the binocular.

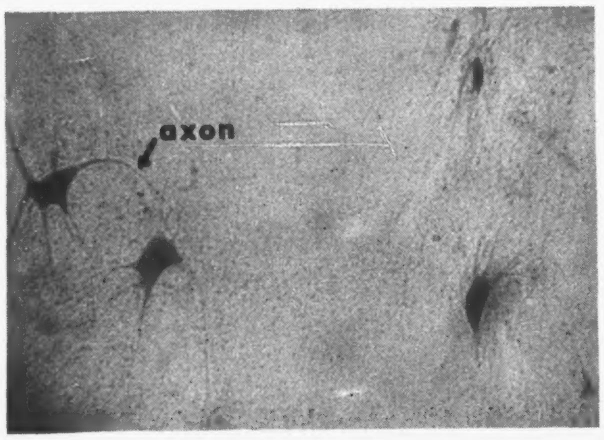


Fig. 15.12 Smear of spinal cord of ox, low power. Both neurons and neuroglia are shown.

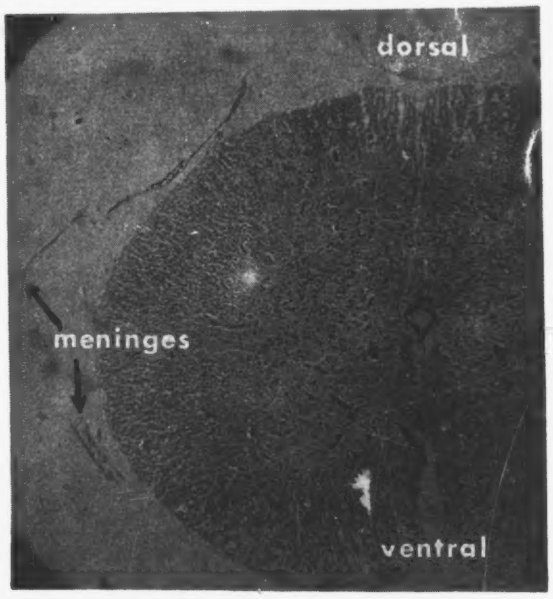


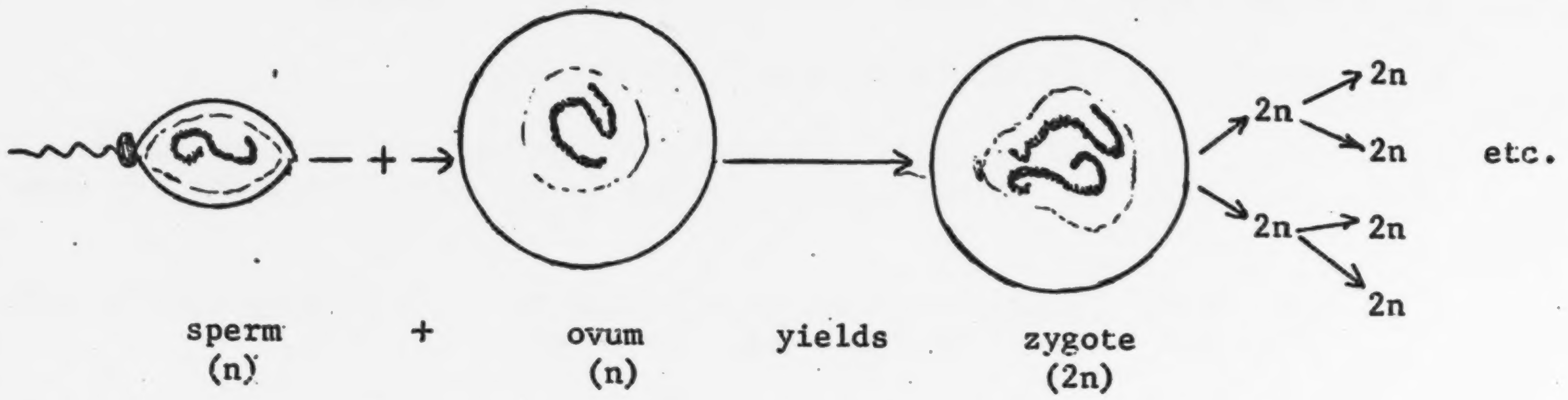
Fig. 15.13 Cross section of frog spinal cord, low power.

LABORATORY WEEK 16:
MITOSIS AND GAMETOGENESIS

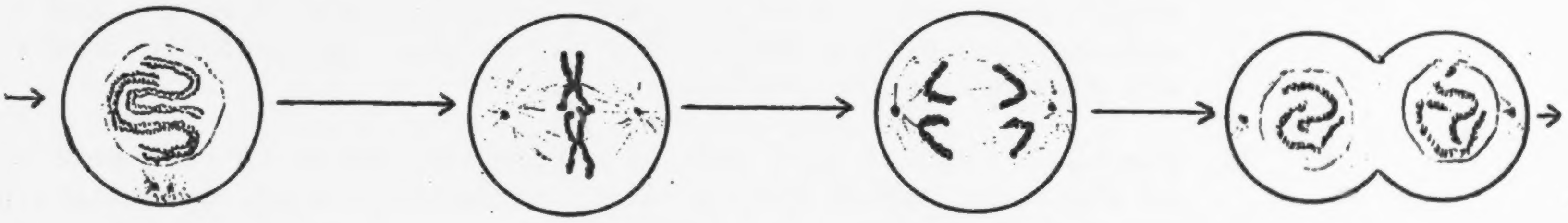
All of the slides in this week's work were made from rapidly-growing tissue that was fixed (killed) while the growth processes were going on, then sectioned and stained to show nuclear material. You are expected to be able both to pick out and to identify various stages in the processes described.

I. MITOSIS

The following diagrams are imaginary, drawn to illustrate an idea, not to show any particular cell. We invent a diplontic living thing that has only one kind of chromosome. In this invented living thing; when the ovum is fertilized by the sperm, each gamete contributes one chromosome ---



--- and, then, when the zygote divides by mitosis, each of the daughter cells gets a copy of each of the chromosomes. If the living thing is a metazoan or metaphyte, many cells are produced and each of the cells has a diploid (2n) nucleus that is identical to each other one. If we examine one of these cells as it divides by mitosis:



Prophase: when chromosomes first become visible, they look double. (4n)

Metaphase: double chromosomes line up on the equator. (4n)

Anaphase: identical chromosomes are pulled apart, move to cell's poles. (2n) (2n)

Telophase: nuclear membranes re-form, chromosomes lengthen, cytoplasm divides (2n) (2n)

During interphase (when the cells are not dividing), the chromosomes become so long and slender that they are no longer visible as strands. It is probably during this time that the chromosomes duplicate themselves, and are thus double for the next prophase.

A. MITOSIS IN ONION ROOT TIP - SLIDE #20

Orient yourself using Figures 16.1 and 16.2. Find the area just behind the root cap where the cells are rapidly dividing. Notice that even the very young cells have a cell wall secreted around them, and that all the cells are oriented longitudinally to the root. Also notice that in telophase, the cytoplasm is divided by a cell plate that grows from the center of the spindle to the outside. Plant cells have no centrioles. Find, and be able to identify, all the stages listed below in C.

B. MITOSIS IN WHITEFISH BLASTODISC - SLIDE #21

Orient yourself using Figures 16.3 and 16.4. All the cells in this slide are in rapid division, since this is a young embryo. None of these cells have cell walls (why?), and the cells are not oriented in any one direction. In telophase, the cytoplasm divides by constriction. Animal cells have centrioles visible. As above, find and identify all stages.

C. THE GENERAL PROCESS IN METAPHYTA AND METAZOA

1. Non-dividing cell. Nuclear membrane present, nucleus appears granular. Single centriole may be visible, if the section went through it.

2. Early prophase. Shortened chromosomes in a tangled mass inside the nuclear membrane. Centriole may be dividing.

3. Late prophase. Chromosomes should appear double in some places. Centrioles should have moved to poles. The end of prophase is marked by the disappearance of the nuclear membrane.

4. Metaphase. The fibers of the spindle between the poles of the cell have now appeared, and the chromosomes become arranged on the equatorial plane of the spindle. If there is not enough room on the plane, then at least the centromere is on it, and and the ends of the chromosomes may dangle. The spindle fibers are attached to the centromere.

5. Anaphase. One of each pair of chromosomes moves to each pole of the cell, making nuclear division exactly equal, quantitatively and qualitatively.

6. Telophase. Chromosomes lengthen and disappear, nuclear membranes form around them. The cytoplasm divides, differently in plants and animals.

NOTE: None of the forms of living things taken up in this week's work have only one kind of chromosome, such as we used in the imaginary form on the previous page. In the forms studied here, onion n=8, whitefish n= about 25, Ascaris n=2, salamander n=12.

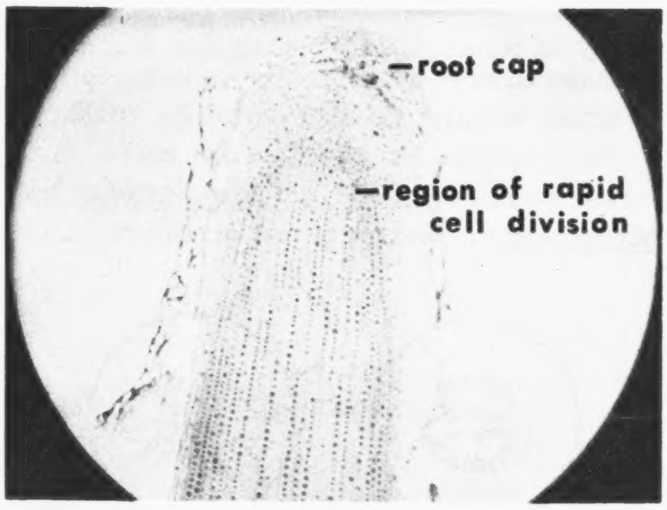

root cap
region of rapid cell division

Fig. 16.1
Longitudinal section of onion root tip, low power.

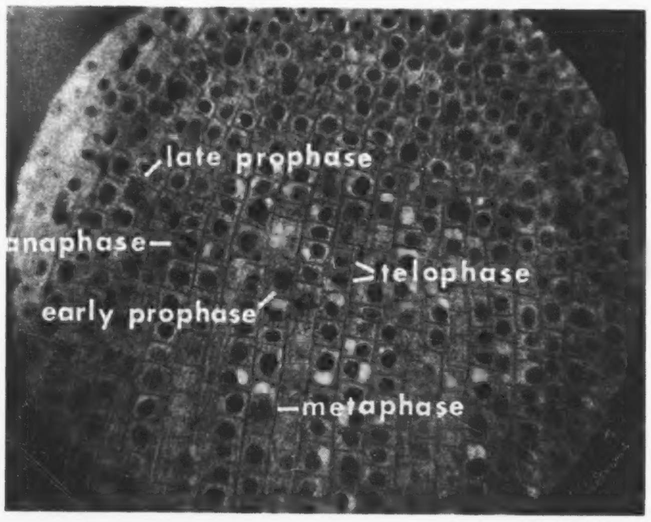

late prophase
anaphase
telophase
early prophase
metaphase

Fig. 16.2
Onion root tip, high power.

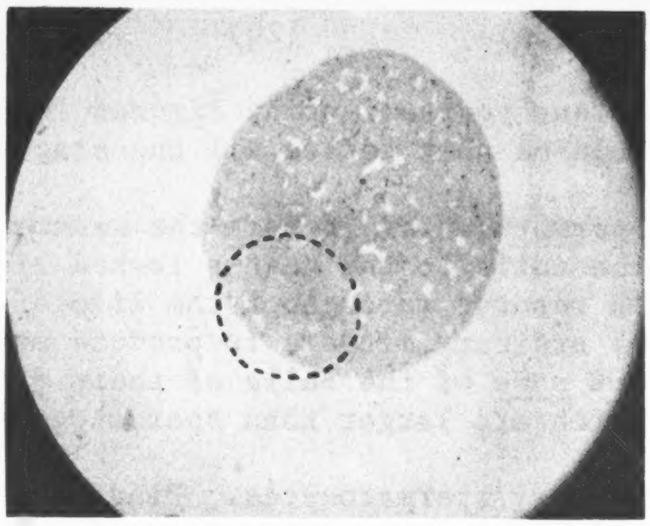


Fig. 16.3
Section of whitefish blastodisc, low power. Area in dashed circle is seen in high power below.

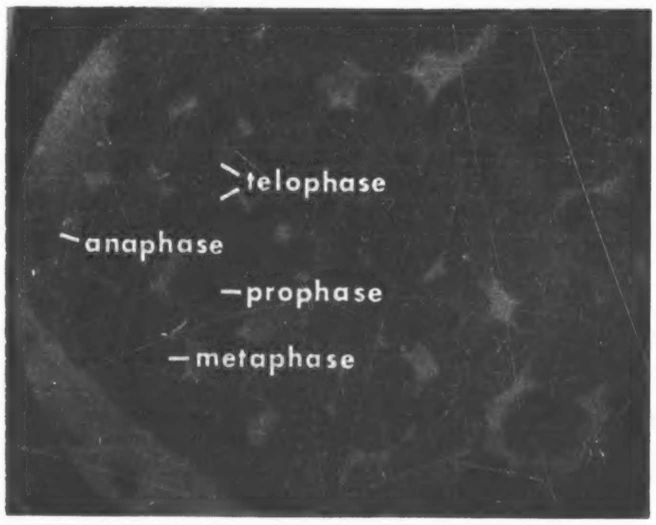
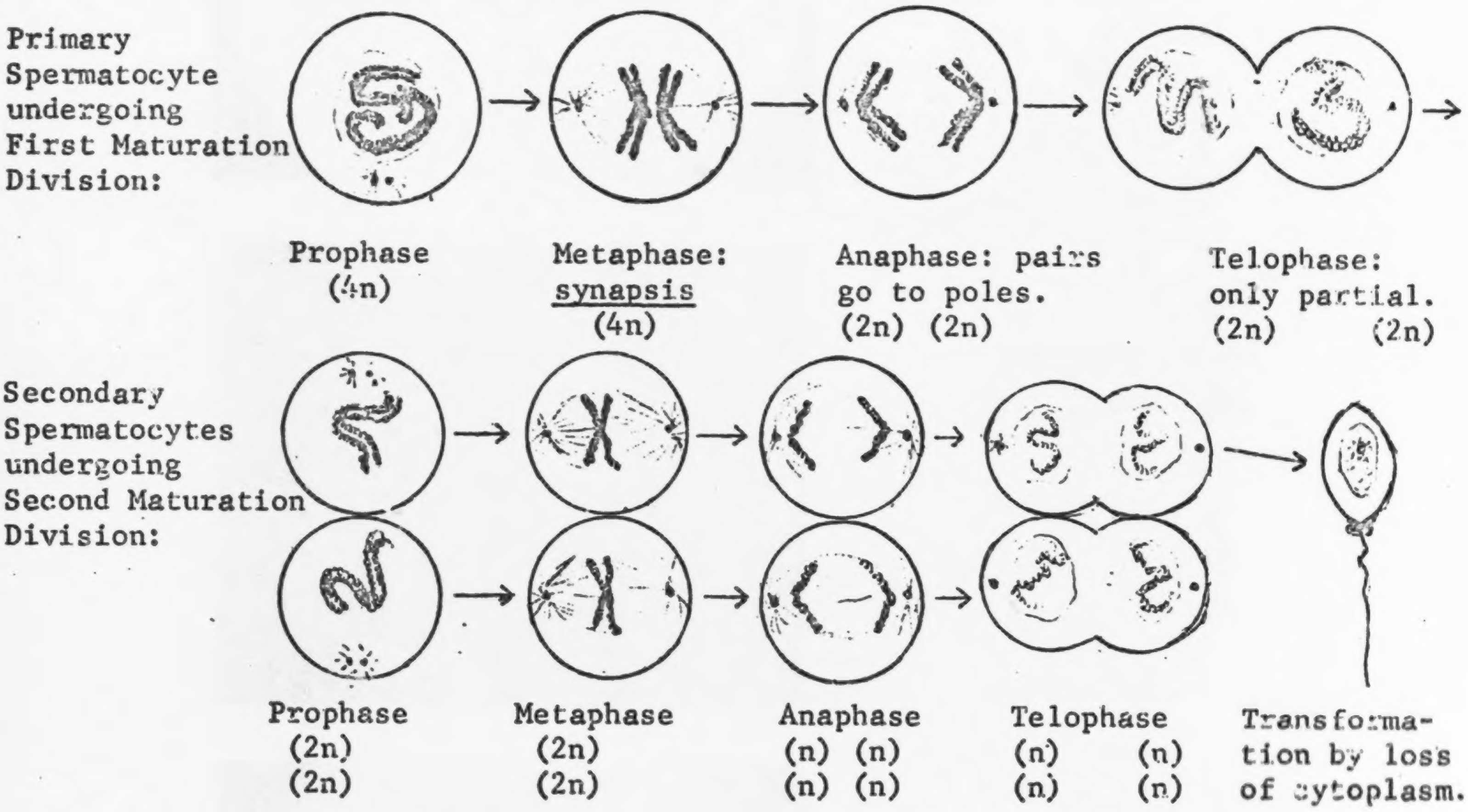

telophase
anaphase
prophase
metaphase

Fig. 16.4
Whitefish blastodisc, high power.

II. SPERMATOGENESIS IN SALAMANDER TESTIS

Return to our imaginary diplontic living thing with only one kind of chromosome. There must be some means to get the 2n number of every cell down to a single n in the gametes (sperm or ova). In male animals, this happens within the testis. Special cell called spermatogonia divide rapidly to produce primary spermatocytes, which then produce sperm in a way like the following diagram:


Primary Spermatocyte undergoing First Maturation Division:
Prophase (4n)
Metaphase: synapsis (4n)
Anaphase: pairs go to poles. (2n) (2n)
Telophase: only partial. (2n) (2n)
Secondary Spermatocytes undergoing Second Maturation Division:
Prophase (2n) (2n)
Metaphase (2n) (2n)
Anaphase (n) (n) (n) (n)
Telophase (n) (n) (n) (n)
Transformation by loss of cytoplasm.

SLIDE #22. Orient yourself using Figures 16.5 and 16.6. Under high power you should be able to see all the stages:

1. Spermatogonia. Before the salamander became sexually mature, the cells in the testis looked like these, and some are kept in reserve throughout the life of the animal. They can divide by ordinary mitosis to produce more spermatogonia, indefinitely. But some of the cells of their division become primary spermatocytes, which are larger than spermatogonia.

2. Primary spermatocytes. Find prophase, metaphase, anaphase, and telophase. The last merges immediately into prophase of the second maturation division..

3. Secondary spermatocytes. Find prophase, metaphse, anaphase, and telophase.

4. Spermatids. After the last nuclear division, the cells wait for a time, and then begin lengthening the nucleus and casting out excess cytoplasm.

5. Mature sperm. Nothing more occurs in nuclear division, but changes in cytoplasm produce head, middle piece and the flagellate tail.

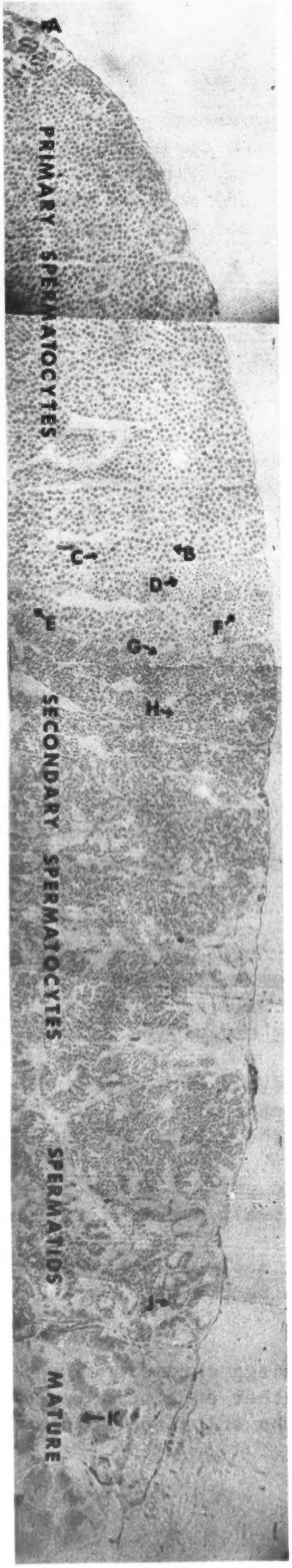
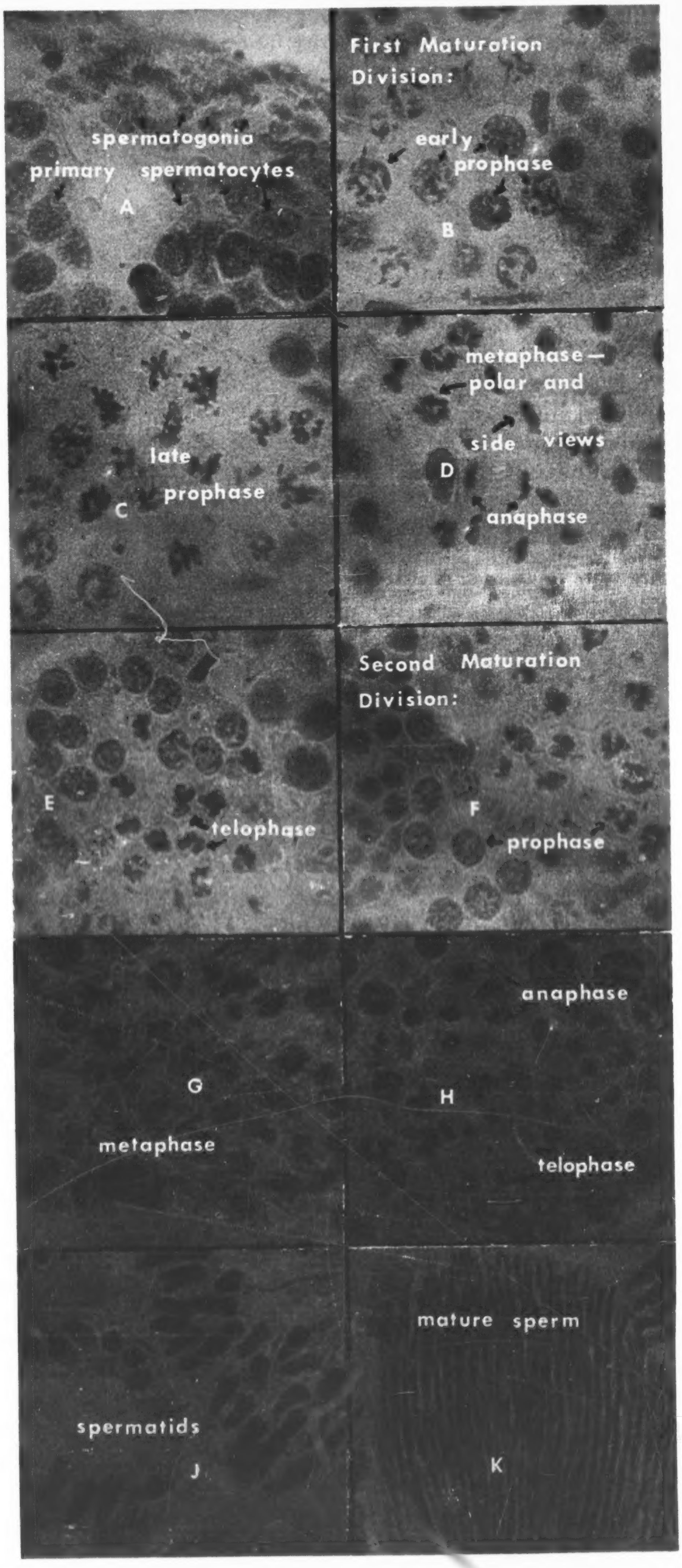

A
PRIMARY SPERMATOCYTES
C
B
D
E
F
G
H
SECONDARY SPERMATOCYTES
SPERMATIDS
J
MATURE
K

Fig. 16.5 Salamander testis, low power.

Fig. 16.6 Stages in spermatogenesis, salamander, oil immersion.


spermatogonia
primary spermatocytes
A
First Maturation Division:
early prophase
B
late prophase
C
metaphase — polar and side views
anaphase
D
E
telophase
Second Maturation Division:
F
prophase
G
metaphase
anaphase
H
telophase
spermatids
J
mature sperm
K

III. OÖGENESIS IN ASCARIS UTERUS

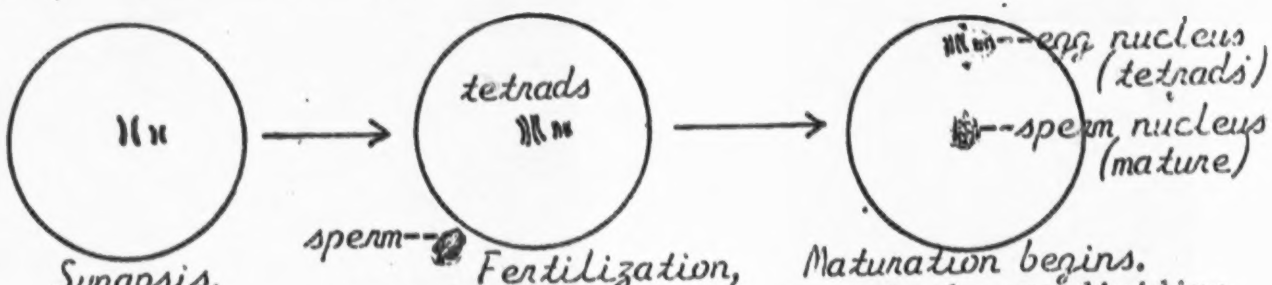
Nuclear processes in oogenesis are just like those in spermatogenesis: synapsis at metaphase of the first maturation division, two divisions producing four cells, each with the n number of chromosomes. The difference is in the cytoplasm: one of the cells gets all the yolk and most of the cytoplasm, and the other three cells become polar bodies and die. Check the diagrams on page 16-4 for nuclear processes.

SLIDE #23. Orient yourself using Figures 16.7 - 16.8. The slides are composites, containing pieces of the uterus of a female Ascaris worm with eggs at different stages of development. Moving from one side of your slide to the other will take you progressively through older stages of development.

When released from the ovary, Ascaris eggs lack a shell, and are blocked from further development until they are fertilized by a sperm. As soon as the sperm penetrates, an egg shell is secreted around the egg, and the egg nucleus moves to the sides of the egg where it undergoes its maturation and casts out 3/4 of its nuclear material in the polar bodies. Find the following:

1. Sperm entrance. Sperm are black and wedge-shaped. No egg shell.

2. Metaphase, first maturation division. The sperm nucleus, having already undergone maturation, has taken over the center of the ovum. The two kinds of chromosomes of the egg nucleus are lined up in two tetrads at the edge. The tetrads are the result of each of a pair of synapsed chromosomes dividing into two.



3. Anaphase, first maturation division. The two tetrads are dividing into two pairs of dyads. One pair of dyads will go to the first polar body, the second will stay in the egg and divide again.

4. Second maturation division. The egg's two dyads divide again immediately into two pairs of monads. One pair of monads will go to the second polar body, the other is now the mature egg nucleus.

5. Maturation completed. As soon as the second polar body is formed, both the egg nucleus and the sperm nucleus swell and move together.

6. Mature ovum. The sperm nucleus and the egg nucleus look identical, and are called pronuclei. They are undergoing prophase of the first cleavage.

7. Metaphase, first cleavage mitosis. The two egg chromosomes and the two wperm chromosomes become mixed together on a spindle, and look like ordinary mitosis as observed in the onion root tip and the Whitefish blastodisc.

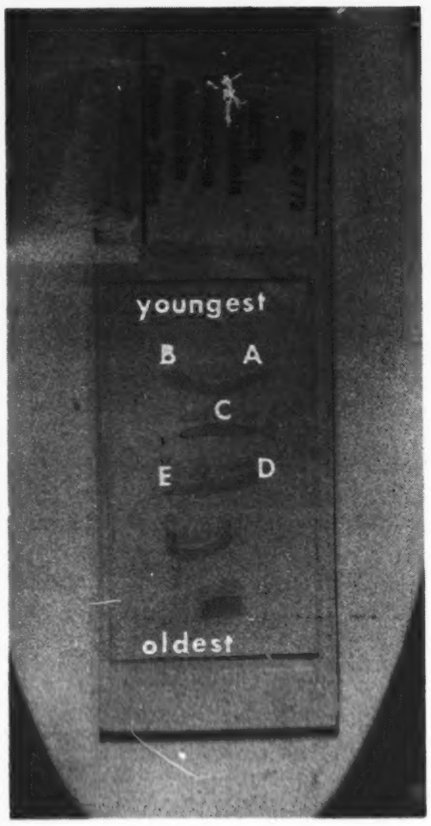

youngest
B
A
C
E
D
oldest

Fig. 16.7
Slide used for
Ascaris oögenesis
study, x1.
Letters show where
high power views
were taken.

Fig. 16.8 Oil immersion views of stages of maturation of Ascaris ova.

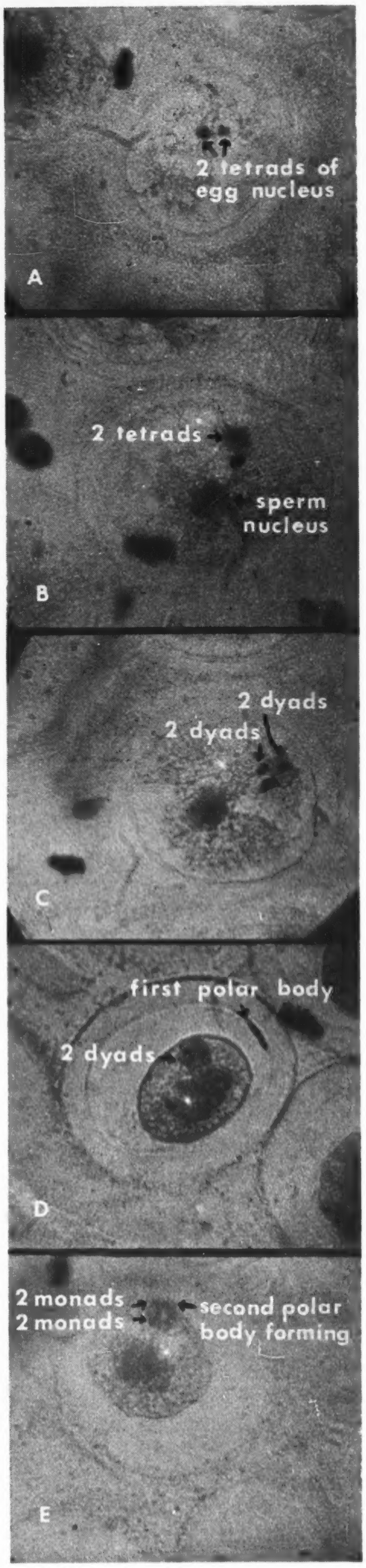
A. Primary oöcyte, unfertilized, before 1st maturation division.

B. Primary oöcyte after fertilization. Metaphase of 1st maturation division.

C. Primary oöcyte. Anaphase of 1st maturation division.

D. Secondary oöcyte. Metaphase of 2nd maturation division.

E. Secondary oöcyte. Anaphase of 2nd maturation division.


2 tetrads of egg nucleus
A
2 tetrads
sperm nucleus
B
2 dyads
2 dyads
C
first polar body
2 dyads
D
2 monads
2 monads
second polar body forming
E

LABORATORY WEEK 17:
METAZOAN EMBRYOLOGY

I. BACKGROUND

Embryology is the study of embryos. Although there is some disagreement among biologists as to the exact meaning of the term "embryo", it is generally agreed that the embryo begins with a fertilized ovum, and remains an embryo until it is more or less ready to feed itself and in other ways be independent of its parent and of stored food materials. Since the embryo begins with a fertilized ovum, its nucleus is made up of equivalent numbers and kinds of chromosomes from both the mother and the father. The nuclei of the ovum and sperm are the material bridges between the generations.

Last week you learned to identify the different stages in the maturation of sperm and ova, and you saw in Ascaris the fertilization of the ovum by the sperm. This week you will study the end of the fertilization process in Ascaris, observing the fusion of the nuclei in the ovum. Then you will look at ova of the starfish, to see the cleavage (= division) of an ovum that has very little yolk in the cytoplasm. Finally you will examine selected stages in the development of frog ova, to get an idea of how vertebrate embryology looks; and you will see some of the complications brought about by large accumulations of yolk material in the ovum's cytoplasm.

II. THE FUSION OF THE NUCLEI

What happens to the nucleus of a fertilized ovum (zygote).

Continue with the older stages of Slide #23
As soon as the second polar body is formed, the egg nucleus has reduced its chromosome complement to the n number, and the ovum is mature. Both the egg nucleus and the sperm nucleus go immediately into prophase of the first cleavage division. The nuclei swell, move toward each other, and at the end of prophase the nuclear membranes disappear and the chromosomes mix together in a single diploid mitotic figure (see Fig. 17.1).

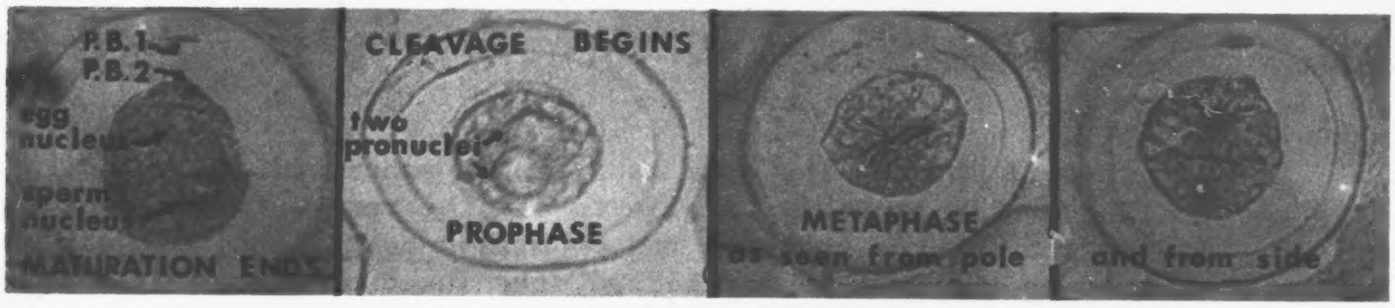


Fig. 17.1 End of maturation and beginning of cleavage, Ascaris eggs.

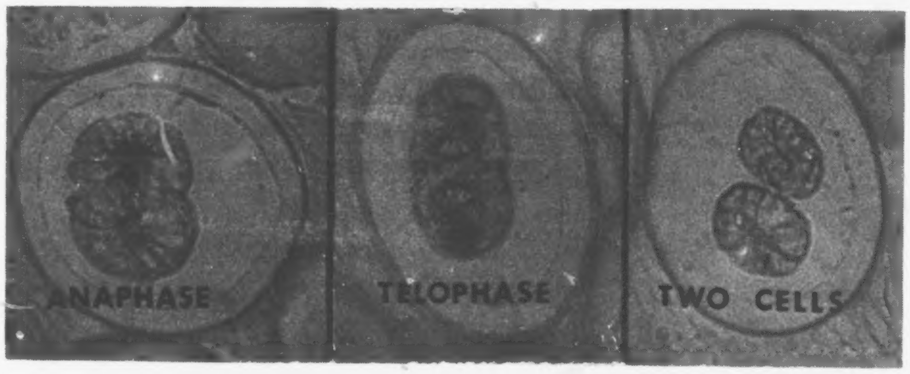


Fig. 17.2 Cytoplasmic cleavage in Ascaris embryos.

III. CLEAVAGE OF THE CYTOPLASM

What happens to the cytoplasm of the zygote during cleavage of the embryo.

A. IN ASCARIS. Continue with the oldest stages of Slide #23.

After the fusion of the two nuclei in metaphase of the first cleavage division, the nuclear process is ordinary mitosis such as you studied in the onion root tip and in the whitefish blastodisc (See Fig. 17.2).

B. IN STARFISH. Use Slide #51, and orient yourself with Fig. 17.3.

In this slide, starfish embryos of all stages of development have been collected from sea water where they have been developing, have been stained various colors, and then put together as whole mounts on slides. The different colors of the embryos have no significance, aside from helping you to tell one embryo from an adjacent one. Different stages of embryos are all mixed together. Notice that while the number of cells doubles with each division, the total volume of cytoplasm remains the same. Thus as cleavage continues, the size of each cell diminishes. DO NOT USE HIGH POWER ON THESE SLIDES.

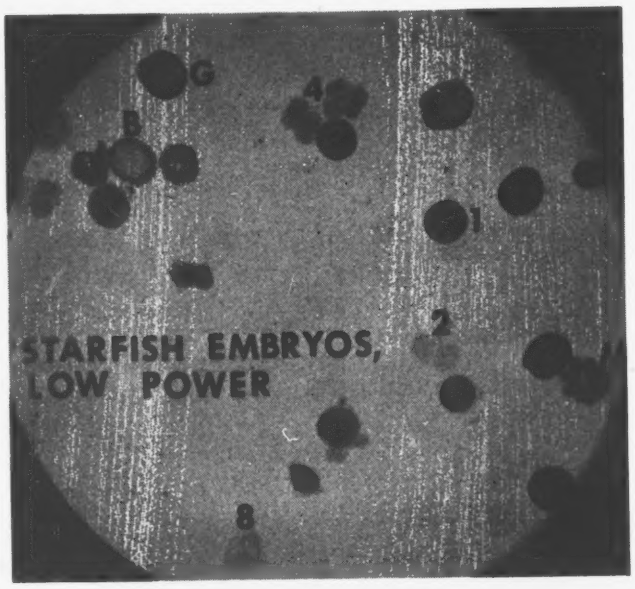


Fig. 17.3
Starfish embryos, low power.
Labels refer to 1-cell, 2-cell, 4-cell, 8-cell, Morula, Blastula and Gastrula.

1. Single-cell Stage. The ovum is a single spherical cell, and in it you will probably be able to see the very large nucleus. If you can see the nucleus, the ovum is unfertilized; because as soon as the ovum is fertilized, the fused nucleus swells up and becomes invisible, while it goes through mitosis (the stain used here does not stain chromosomes). You should not expect to see nuclei in any of the cells of future stages. Identify the cytoplasm of the ovum, called ooplasm because of the large amount of yolk material in it. Find the cell membrane and the vitelline membrane around it.

2. Two-cell stage. As soon as the ovum becomes fertilized, the nucleus divides and then the öoplasm divides into two nearly-spherical cells that lie next to each other, both inside the vitelline membrane.

3. Four-cell stage. Each of the above two cells divides into two again, making four cells lying inside the vitelline membrane.

4. Eight-cell stage. Each of the four cells divides into two again, making eight cells. You will find these lying at different levels, four on top of the other four, so that they can be seen only by focussing up and down with the fine adjustment of the microscope. Compare the relative sizes of the cells. Notice that one quartet is larger than the other. These cells contain more yolk material, and are called the vegetative quartet. The smaller quartet is called the animal quartet. The center of the vegetative quartet is the vegetative pole of the embryo; the center of the animal quartet is the animal pole.

5. Morula. The cells continue dividing -- those at the animal pole more rapidly than those at the vegetative pole. Morulas look somewhat like raspberries. A space begins to appear at the center of the morula, called the cleavage cavity. Notice that as cleavage continues, the cells get smaller and the cleavage cavity increases in size. Morulas have little organization, but at some time during this division period, organization takes place, and the cells begin to look like a layer or membrane, instead of separate cells.

6. Blastula. This is a hollow ball, in which it will be difficult to distinguish individual cells. The cleavage cavity is larger than it is in the morula. The wall of this hollow ball is thicker on the vegetative pole side than it is on the animal pole side. By careful focussing, make sure that you see this difference in thickness.

7. Beginning of gastrulation. You will find some blastulas that are no longer spherical, but instead are getting flat on the vegetative pole end. Other specimens will be found in which the vegetative pole cells are actually pushing inside the cleavage cavity toward the animal pole, like pushing in the side of a soft hollow rubber ball. These stages show the beginnings of gastrulation.

8. Gastrula. The pushing-in process continues until there is formed a true gastrula. You should find many gastrulas in your slide, at various stages. Draw a typical one. Notice how the cleavage cavity is made smaller with the pushing-in of the vegetative pole cells, and a new cavity is found in the center of these cells, the archenteron.

(Arch- = primitive; enteron = gut.) This new cavity will become the digestive cavity of the individual. The embryo is lengthening, because the cells of the animal pole are dividing rapidly, and are growing around the more leisurely vegetative cells.
Two layers of cells are produced by the process of gastrulation: on the outside is the ectoderm ("outer skin"), and on the inside, lining the archenteron, is the endoderm ("inner skin"). What kind of cells are each of these made of?

We will not study the embryology of the starfish beyond this point, because the embryos begin to specialize too much toward starfish to be useful in our general study. But up to this point, the starfish demonstrates very clearly the processes of blastulation and gastrulation, which are much more difficult to see in the frog.

IV. FROG EMBRYOLOGY

What happens when cleavage is modified by large amounts of yolk.
What happens in later development of a vertebrate.

Materials: 1 dish of mixed frog embryos
Demonstrations: models of frog embryology and slides of sections through embryos.

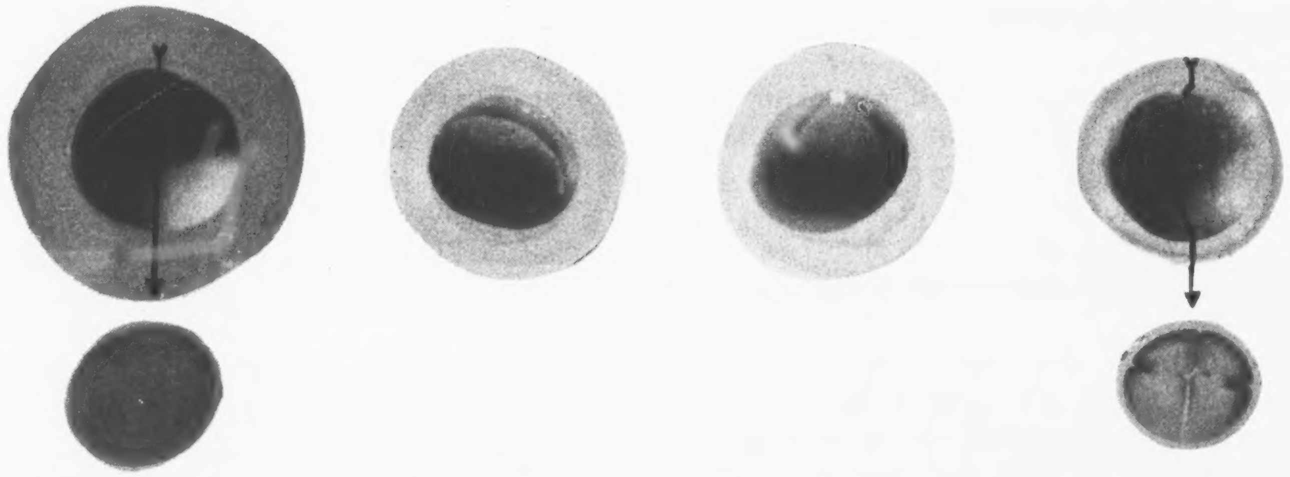
In this animal, the ova are fertilized as they leave the body of the mother. The ovum is much larger than that of the starfish, because it contains large amounts of yolk material as nutrition for the developing embryo. The yolk is heavy and inert, and is reluctant to enter into the processes of embryology readily, and so the parts of the vegetative pole that contain large amounts of it will fall behind the processes at the animal pole. In these eggs, the processes of cleavage and gastrulation will be highly modified.

Use the dish of mixed frog embryos: Look at them with a binocular microscope, with a light from the side. Please do not touch the embryos with anything except a soft camel's hair brush, since they are very brittle. The dish contains about 20 embryos: 12 are spherical, 8 are elongated. The spherical ones are the young stages.

A. CLEAVAGE: single cell through blastula. Find the stages, and examine the demonstrations as illustrated in Fig. 17.4.

All are spherical embryos, and are very obviously divided into two hemispheres: the very dark animal hemisphere containing the animal pole at its center, and the light-colored vegetative hemisphere containing the vegetative pole at its center. The animal pole is the future head end of the embryo, and the vegetative pole is at the future anal end.

Note: All photographs of frog embryos are x20. Stages are after Shumway as listed in Rugh, Roberts, Experimental Embryology, edition 3, 1962, pp. 56-63. Ages given are approximate number of hours after fertilization grown at 18° C.

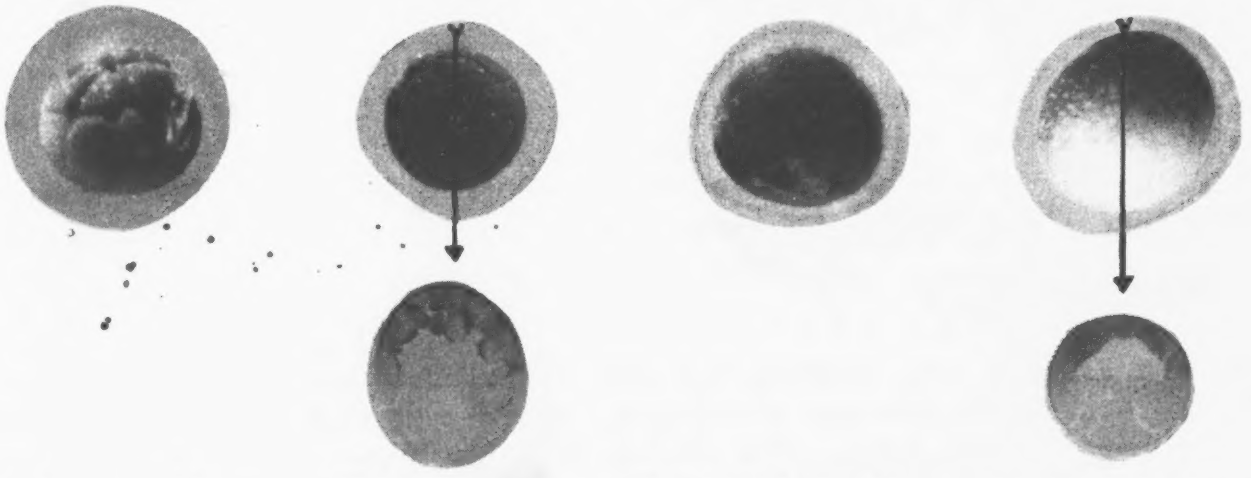


One cell,
stage 2,
1 hour.

Two cells,
stage 3,
3.5 hours.

Four cells,
stage 4,
4.5 hours.

Eight cells,
stage 5,
5.7 hours.



Sixteen cells,
stage 6,
6.5 hours.

Thirty-two
cells, stage
7, 7.5 hours.

Early blastula,
stage 8,
16 hours.

Late blastula,
stage 9,
21 hours.

Fig. 17.4 Cleavage, single cell through blastula. Cross-sections are shown below the whole embryos, and these are set up on demonstration.

Notice that the blastula is larger than the single cell, due to the space taken up by the cleavage cavity inside.

In the demonstration of the cross-section of a blastula, find the cleavage cavity which is displaced toward the animal pole, and find the large yolk-filled cells of the vegetative pole.

B. GASTRULATION

Find the stages, and examine the demonstrations, as illustrated in Fig. 17.5. Even in the dorsal lip stage, the light-colored yolk-filled cells of the vegetative pole will take up much less than half of the embryo, and the animal pole cells will cover much more than half of the embryo. This is because the cells of the animal hemisphere are growing downward over the yolk cells.

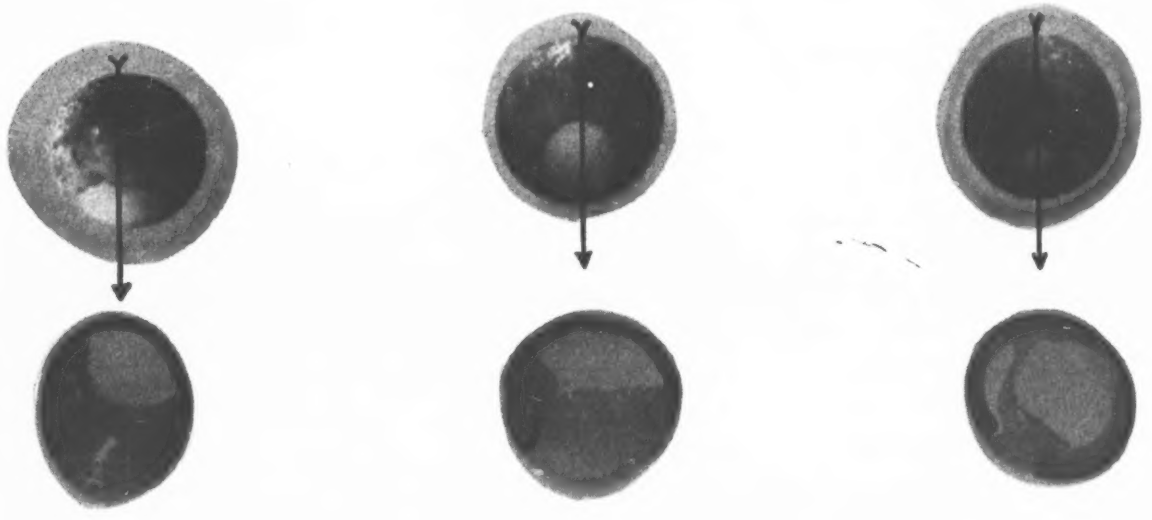
Because of the large amount of yolk, the blastula cannot simply push in to form the gastrula, as in the starfish. The growing-over process is necessary to the process of gastrulation in the frog. The dorsal lip leads all the rest in the downgrowth. It is very dark in color, and lies between the dark and light hemispheres. The cells are growing inward here, forming a double wall. The "yolk plug", in older stages, marks the spot where the future anus of the embryo will be.

In the demonstration of the sagittal section through a gastrula, notice that on the inside of the embryo there are two cavities: the old cleavage cavity and the new archenteron which extends upward from the dorsal lip. The embryo now has two primitive germ layers: the ectoderm covering the outside of the embryo, and the endoderm lining the archenteron. In later stages, the third layer called mesoderm will slide in between the other two, and will obliterate the remains of the cleavage cavity.

C. NEURULATION

Find the stages, and examine the demonstrations, as illustrated in Fig. 17.6. These embryos are beginning to lengthen along the future anterior-posterior axis. The dorsal side is marked by two definite ridges or folds, called neural folds, which grow at the sides of a flattened region, the neural plate. Both folds and plate are the beginning of the central nervous system of the embryo. At the anterior end of the embryo the folds meet in a broad curve, marking the future brain. At the posterior end the folds meet again, around the yolk plug or future anus of the embryo (you may find that the yolk plug has entirely disappeared at this stage).

Imagine a section cut across the embryo, perpendicular to the neural folds. The demonstration is such a cut. Find the neural folds and the neural plate. These are made up of special ectoderm cells called neurectoderm. All the rest of the outside of the embryo is covered with ectoderm. The large space on the inside is the archenteron, and is lined with endoderm. Notice that the mesoderm lies between the ectoderm and the endoderm, and it extends nearly around the yolk.

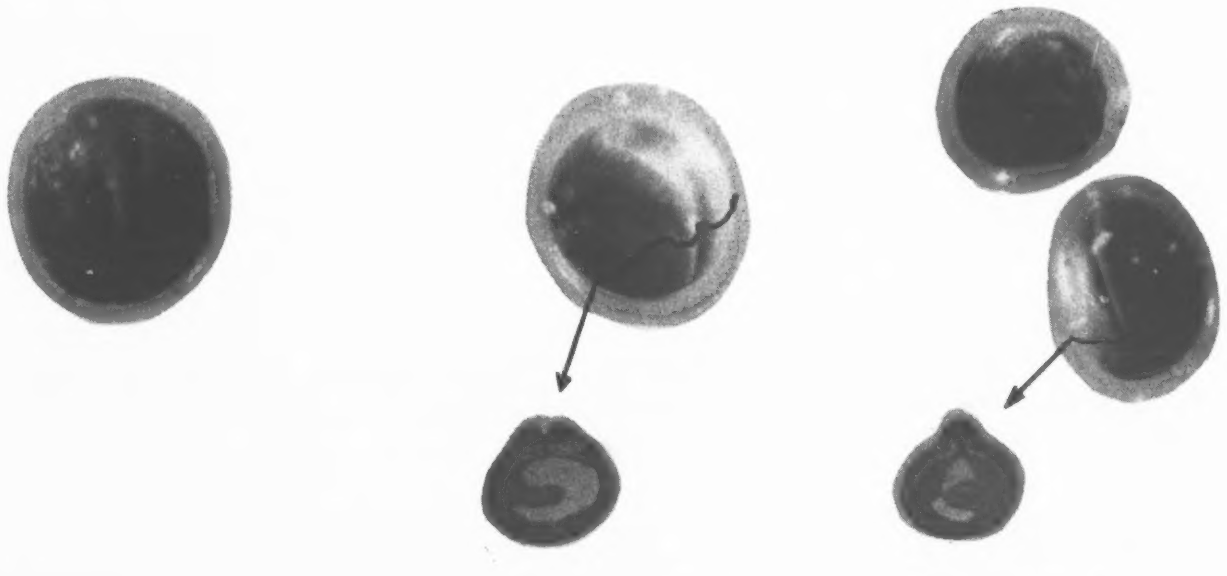


Dorsal lip,
stage 10,
26 hours.

Gastrula,
early yolk plug,
stage 11,
34 hours.

Gastrula,
disappearing yolk plug,
stage 12,
42 hours.

Fig. 17.5 Gastrulation. Cross-sections are below the whole embryos.



Neural plate,
stage 13,
50 hours.

Neural fold,
stage 14-15,
62-67 hours.

Neural tube,
stage 16,
72 hours.

Fig. 17.6 Neurulation. Cross-sections are below the whole embryos.

A special type of mesoderm called the notochord is being set off, just under the neurectoderm.

D. ELONGATION, DIFFERENTIATION, AND HATCHING.

Find the stages, and examine the demonstrations, as illustrated in Fig. 17.7. The tailbud stage is the shortest of the tadpole-shaped embryos. During the formation of the dorsal neural tube, the body has become elongated, and other structures appear rapidly. Head, trunk and tail are distinguishable at this time; the latter as a small dorsal bud which gives this stage its name. Find the future anus just below the tailbud. Anteriorly, notice the thickened region where the gills, eyes and ears will appear. At approximately the gill ridge stage, the embryo usually hatches and begins to feed soon afterward.

Imagine a section across the embryo, perpendicular to the long axis of the body about halfway back. The section, described below, was cut on this line. On the slide are cross-sections from various parts of the body, arranged in order. Choose one from about the middle. Notice that the neurectoderm has formed a tube, the neural tube, and is covered like the rest of the body with ordinary ectoderm. The archenteron has become differentiated, and in the region that you have chosen it is the intestine. It is lined as before with endoderm. Find the mesoderm: it has increased in thickness and extent, and is becoming subdivided. Find the notochord, just under the neural tube.

E. GROWTH AND DEVELOPMENT

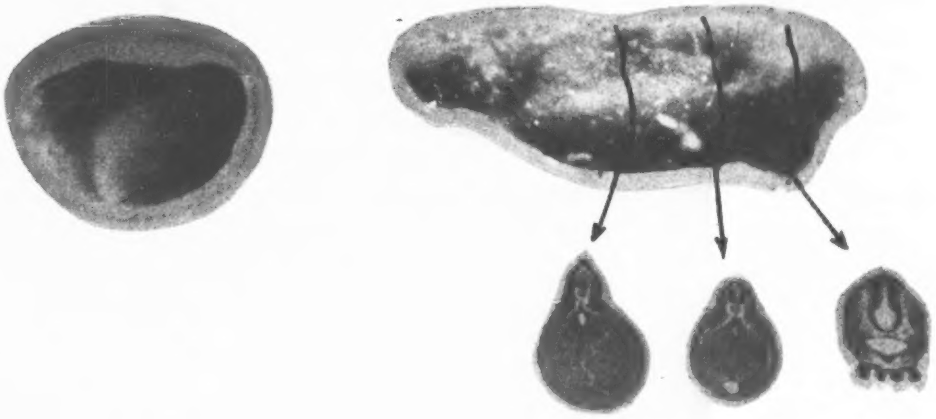
Find the stages, and examine the demonstrations, as illustrated in Fig. 17.8 and 17.9. Since the animals can now feed, you would expect some increase in total mass for the first time. In addition, many organs appear and others change. The tail becomes longer, and is compressed laterally. Fin-like ridges extend from it dorsally and ventrally. The belly of the embryo bulges becuase of stored yolk. The mouth opens, and gills, nostrils and eyes are developing. Notice the V-shaped muscle segments that run down the sides of the body. The anus is at the base of the tail.

The feathery external gills develop laterally, behind the head. Later an operculum grows posteriorly from the head and covers the gills. This operculum remains open to the outside through a spiracle on the tadpole's left side.

F. METAMORPHOSIS

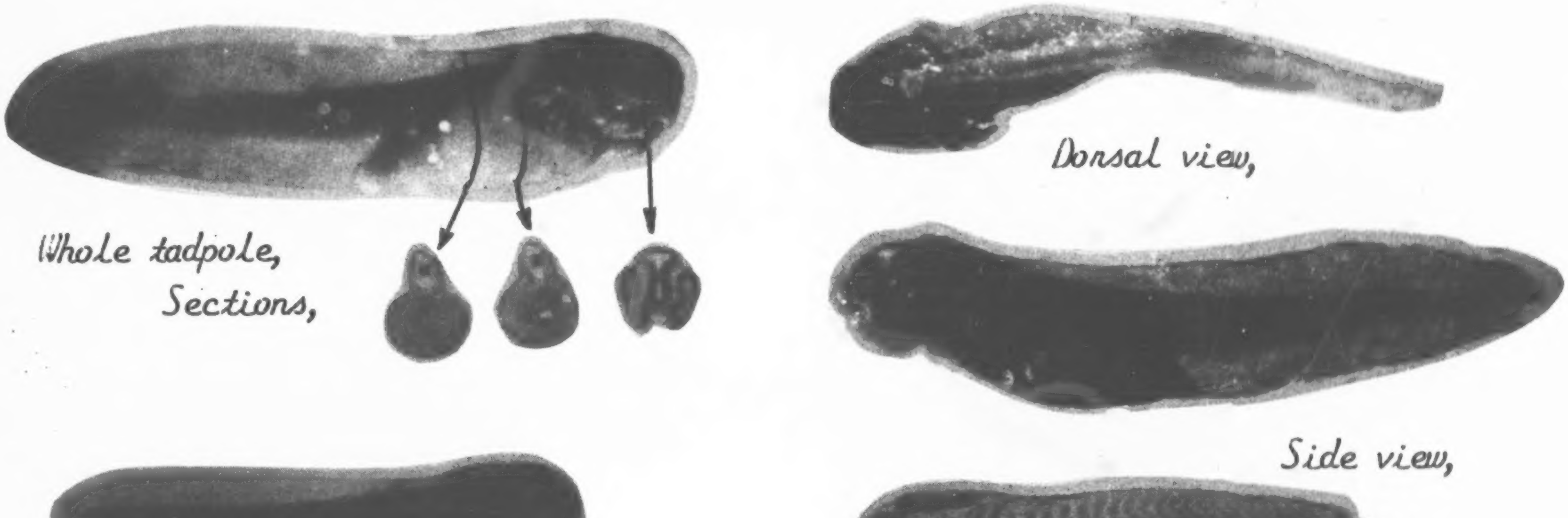
Specimens of older tadpoles are in watch glasses on demonstration. Notice how the hind legs grow out of the body, near the anus. The body begins to change its tadpole shape, in preparation for metamorphosis or change to a land-living animal. Note the belly, enlarged to accommodate the long coiled gut needed by an animal that eats vegetation. Notice also the mouth, adapted to nibbling plant material.

Fig. 17.7
Growth,
differentiation
and hatching.



Tail bud,
stage 17,
84 hours,
3 mm.

Gill ridge,
stage 18,
96 hours,
4 mm.



Whole tadpole,
Sections,

Cleared.

Dorsal view,

Side view,

Sagittal section.

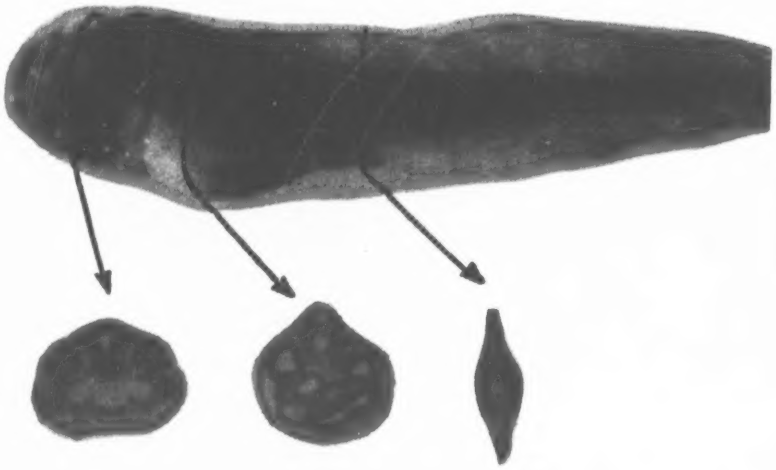
External gills,
stage 20,
140 hours,
6 mm.

Tail fin circulation,
stage 22,
192 hours,
7.5 mm. or more.

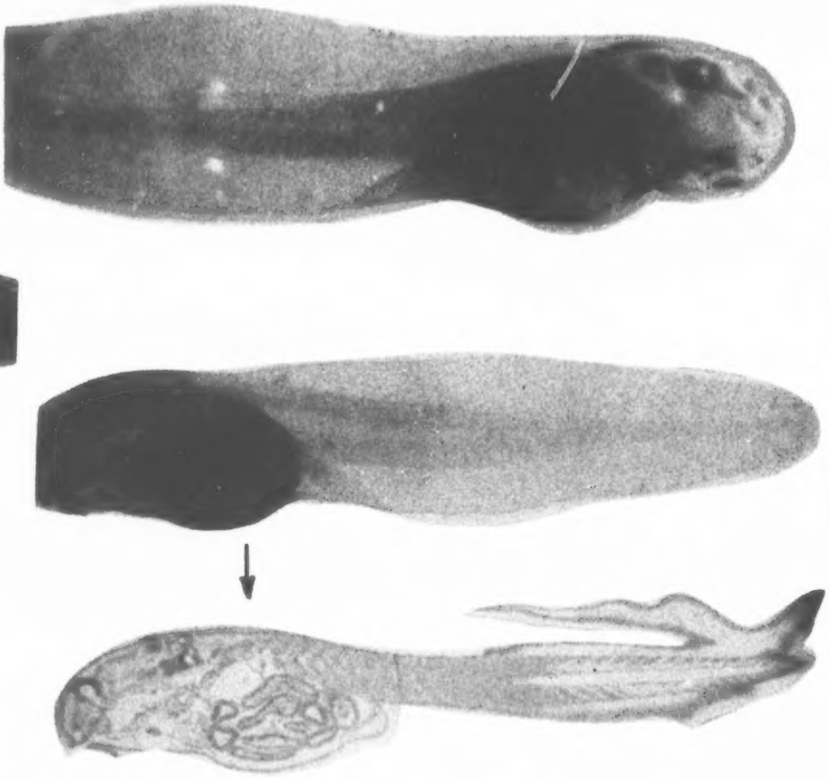
Fig. 17.8 Early growth and development stages of the tadpole.

17 - 10

The tadpole may remain a tadpole for different lengths of time depending on the species -- either one, two or three summers. Metamorphosis from tadpole to frog involves many changes, internal and external, which adapt the animal to life in air and to changes to animal food. Loss of gills, closure of gill slits, development of lungs, changes in the circulatory system, increase in size of the mouth opening, enlargement of the stomach and shortening of the intestine are some of the changes. Note the development of front legs, loss of tail, and general change in body shape.



Opercular fold, stage 23,
over 200 hours, under 10 mm.



Operculum nearly completed, stage 24,
10 mm. or more.

Fig. 17.9 Later growth and development stages of the tadpole.

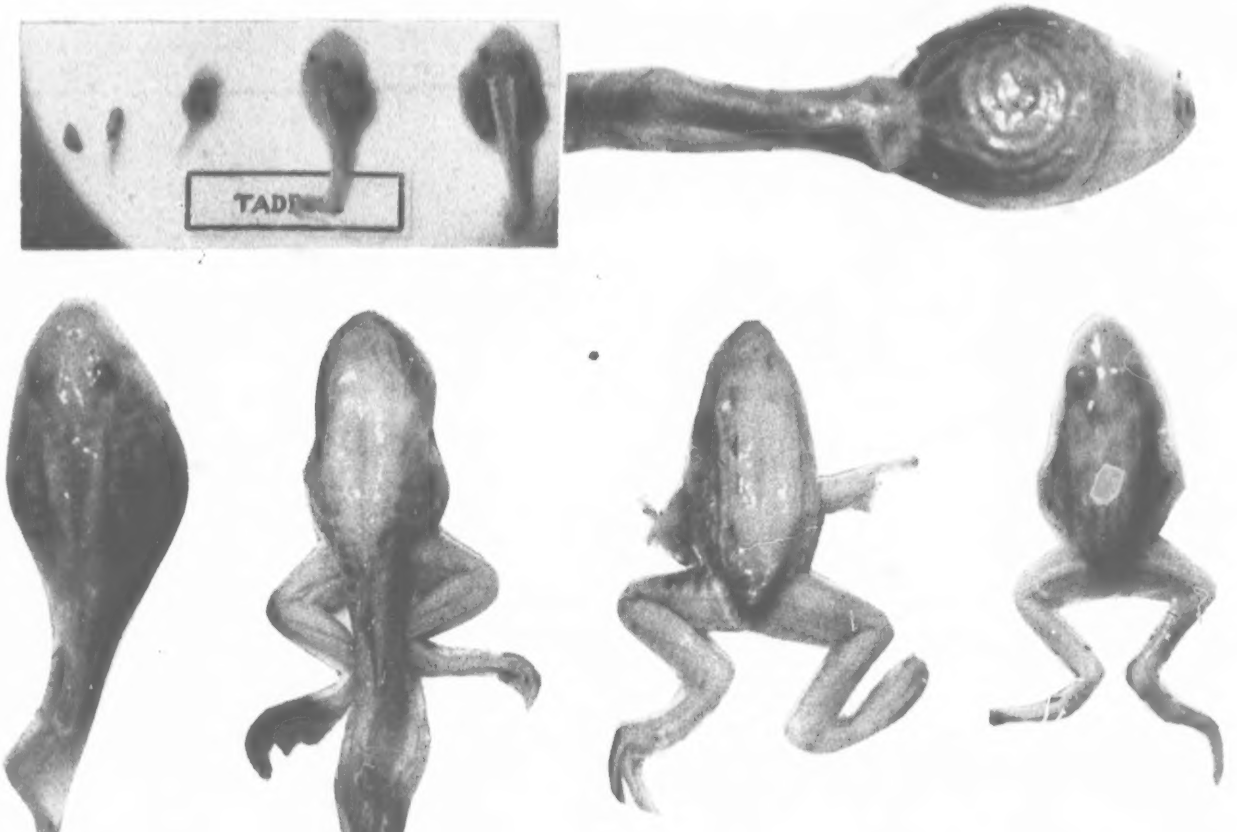


Fig. 17.10 Frog tadpoles, natural size, showing extent of growth
and stages of metamorphosis.

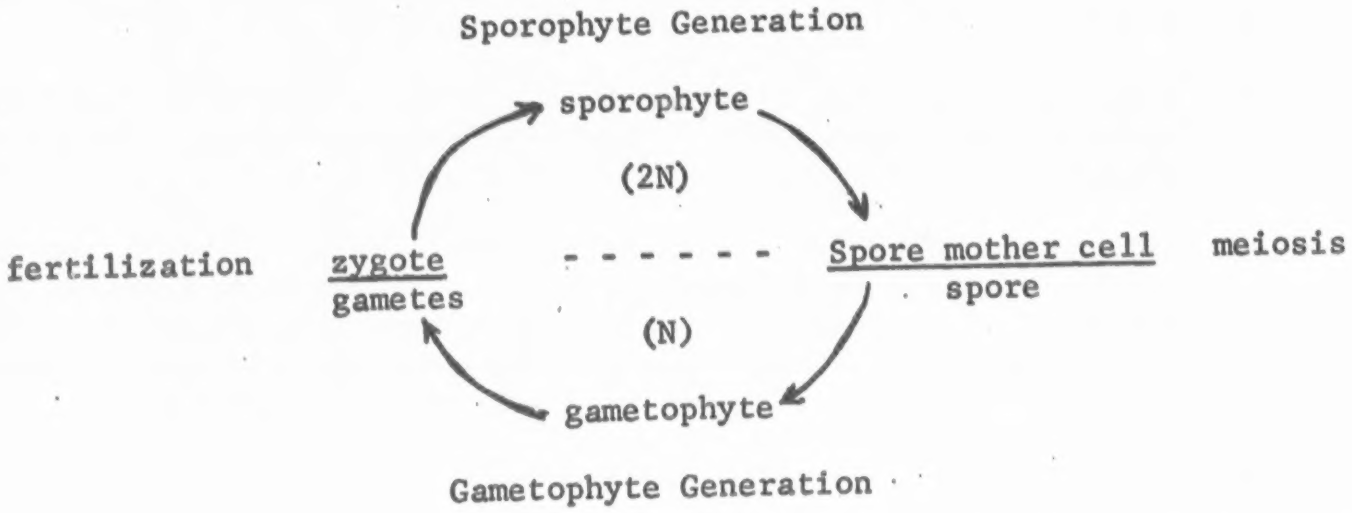
LABORATORY WEEK 18:
NON-FLOWERING METAPHYTES

I. BACKGROUND

You should have learned from the lectures and reading that all green multicellular land plants are included in the kingdom Metaphyta. The Metaphyta includes two phyla, the Bryophyta or mosses and the Tracheophyta or vascular plants. The characteristics of the Metaphyta are mainly those which have evolved in adaptations to the terrestrial habitat, for example, Metaphyta possess specialized:

supporting tissues,
absorbing tissues,
protective coverings,
multicellular sex organs,
a distinct embryonic phase.

Metaphyta also possess a distinct alternation of generations in which a gametophyte (plant producing gametes) generation alternates with a sporophyte (plant producing spores) generation illustrated by the following diagram:



Since metaphyta are diplohaplontic, the reduction division and the production of gametes are separated by a whole haploid generation, called the gametophyte. Compare this with gametogenesis in metazoa.

All of the cells in the mature sporophyte are diploid (2N). When the sporophyte is mature, certain of these diploid cells undergo the process of meiosis (reduction division) which in effect reduces the chromosome number to haploid (from 2N to N). The haploid spores resulting from meiosis are capable of growing into a haploid gametophyte which when mature will bear haploid gametes.

Two gametes (egg and sperm) then fuse in the process of fertilization, resulting in a zygote or the first diploid cell of the sporophyte generation. The zygote then grows into an embryo and eventually into a mature sporophyte. The sporophyte generation begins with the zygote and ends with the production of spore mother cells. The gametophyte generation begins with the spore and ends with the formation of gametes.

II. CULTURE OF MOSS AND FERN SPORES

This work must be carried out at least two weeks in advance, and some observation will be necessary before the eighteenth week. Notice from the previous diagram and discussion, where you are starting in the life cycle. Proceed as follows:

1. From the refrigerator obtain a sterile petri dish containing sterile plant nutrient agar. With a wax pencil line, divide the bottom of the dish into two equal halves.

2. Label one side "MOSS" and the other side "FERN", and add your name and the date clearly. This will be grown in the window, not in your locker, so identification is important.

3. Dip the camelhair brush (provided) lightly into the MOSS spores, shake off most of the spores, and then sow a few on the surface of the "MOSS" side of the petri dish. This is best done by gently tapping the handle of the brush, distributing a small number evenly over the entire half. Do not cross-contaminate the MOSS and FERN brushes.

4. Repeat with the FERN spores on the "FERN" side of the petri dish.

5. Place the dish in the window, cover upwards.

6. Mount a few of each kind of spore in water on slides, cover and examine under high power of the compound microscope. Record your observations of each.

7. The spores should germinate within 10-14 days, although the moss is usually more rapid than the fern. Observe them frequently at the time of germination, keeping good records. If you wait until the surface looks green, you will have waited too long -- some of the most interesting growth will already have taken place.

8. You may observe in either of two ways:

   a. Place the uncovered petri dish directly on the stage of a binocular or compound microscope. Do not use high power of the compound microscope in this way. Figures 18.2 and 18.4 are taken under low power of the compound microscope; 18.3, 18.5 and 18.6 are taken under high power of the binocular microscope.

   b. Using good sterile technique, scrape a portion of the agar containing the germinating spores, and mount in water on a slide. Cover with a coverglass. This slide may be observed under high power of the compound microscope.

9. Each spore, when germinated, can develop into a mature gametophyte plant. Each produces a small filament which soon grows into a mass of filaments. The filaments become more and more organized as the plant grows, and within a short time can be recognized as a moss or fern gametophyte. Conditions on the agar will probably not support the growth of these gametophytes beyond a few weeks, however, because the agar dries out. Addition of a few drops of sterile water occasionally will often help to maintain the cultures.

LABORATORY WEEK 18:
NON-FLOWERING METAPHYTES

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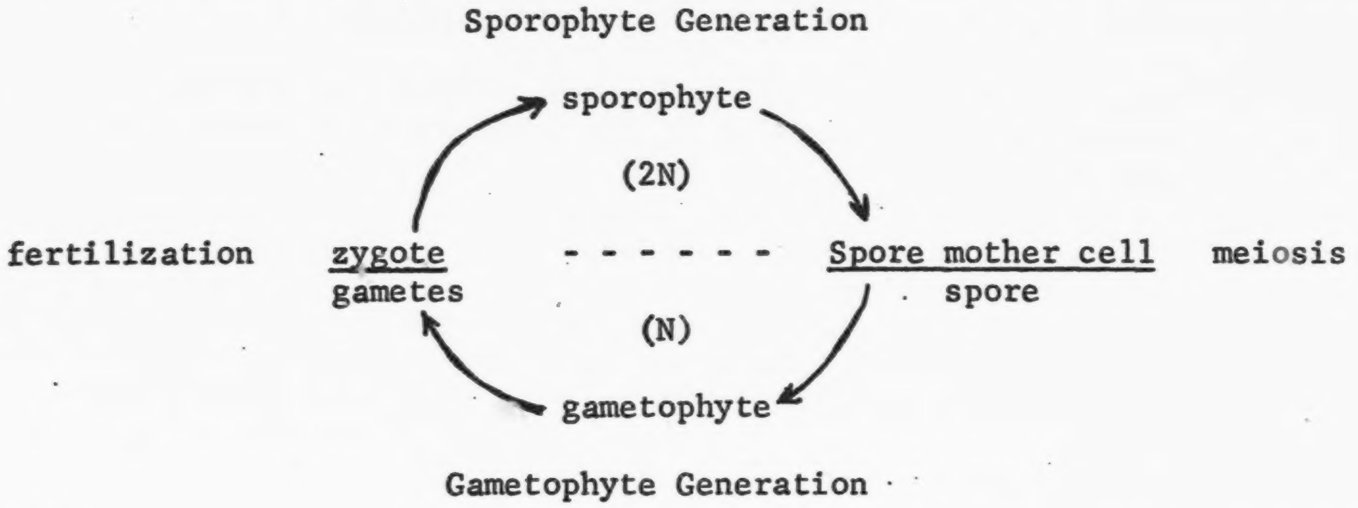

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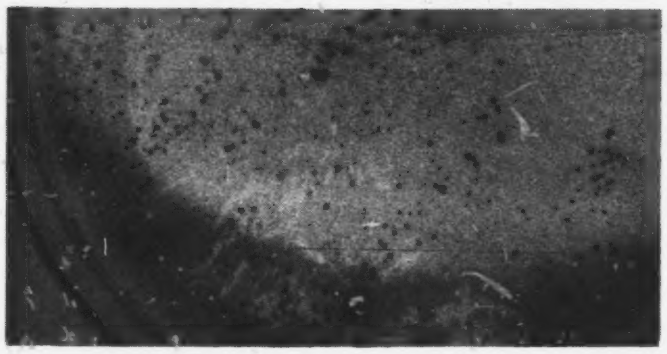


Fig. 18.1 Germinated moss spores on agar, life size.

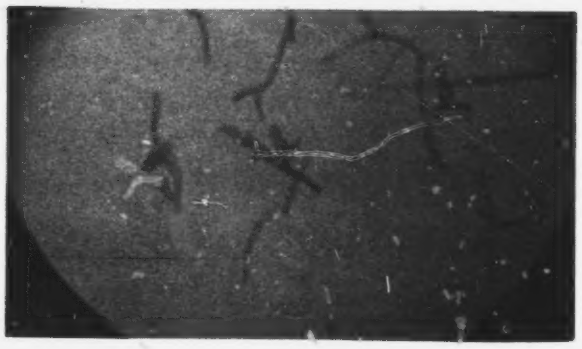


Fig. 18.2 Moss protonemata or germinated spores, x100.

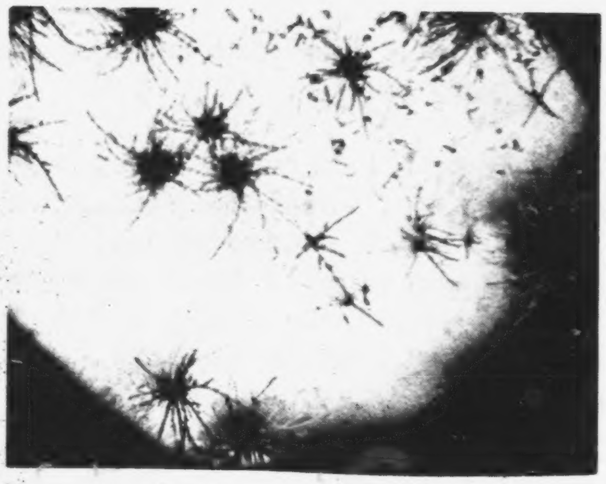


Fig. 18.3 Young moss gametophytes on agar, magnified x20

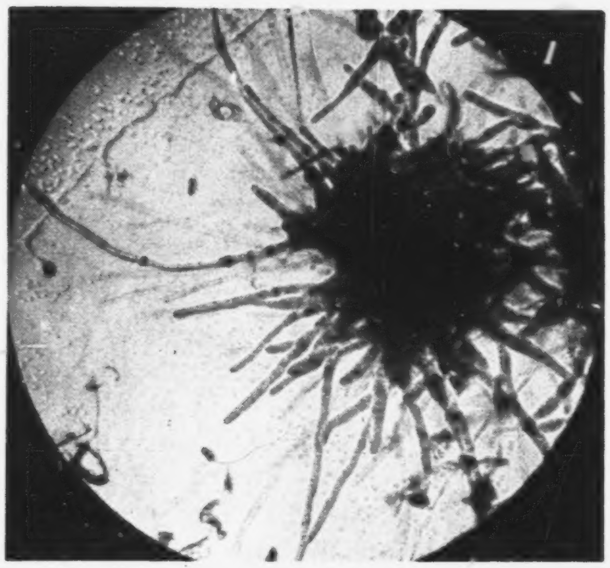


Fig. 18.4 Young moss gametophytes on agar, magnified x100.

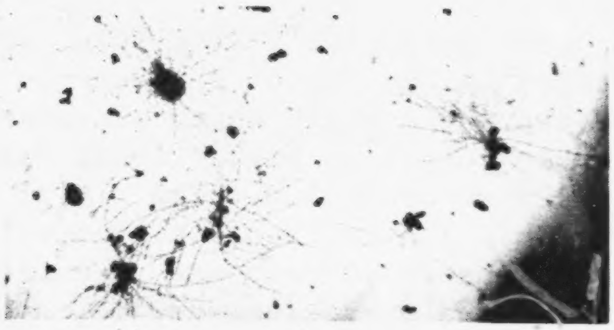


Fig. 18.5 Young fern gametophytes on agar, magnified x20.

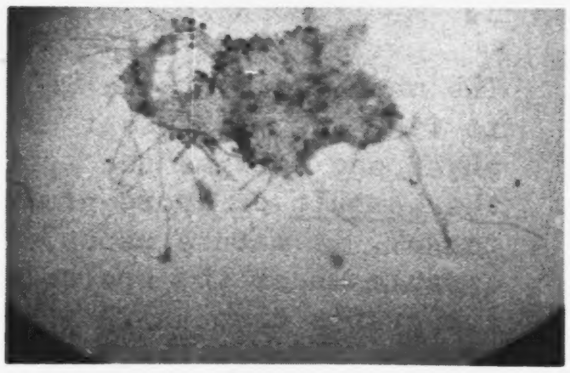


Fig. 18.6 Older fern gametophyte, with antheridia, x20. (Archegonia come later on the same gametophyte.)

10. The young stage of the moss gametophyte is called the protonema. On the protonema small "buds" appear which develop into the mature gametophyte, consisting of "leaves", "stem", and rhizoids. The "leaves" and "stem" lack well-developed conducting tissue and therefore are not considered true leaves and stems. What is the function of the rhizoids? What does the gametophyte produce?

11. The fern spore grows into a filament of cells first and then into a mature heart shaped gametophyte which is seldom more than a centimeter long. Note its rhizoids and general shape. At the base of the heart, on the lower side among the rhizoids you may see atheridia containing sperm, as in Fig. 18.6. Later, near the notch of the heart you may see the necks of archegonia.

III. PHYLUM BRYOPHYTA

Take one preserved specimen each of moss male gametophyte, female gametophyte, and sporophyte, and compare them with Fig. 18.7. These are older than those you can grow on agar, and the gametophytes are sexually mature.

A. GAMETOPHYTES

In life, sperm from the male swim the eggs of the female, and the sporophyte grows from these as a parasite on the female gametophyte.

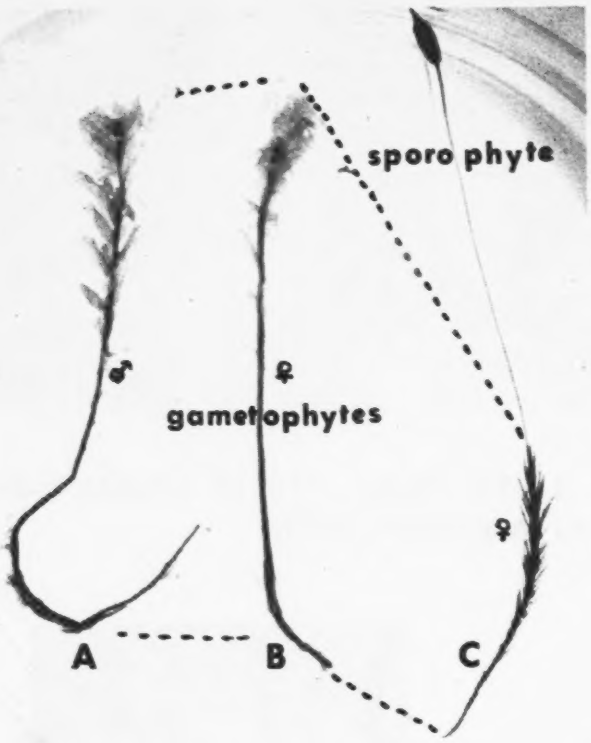
Remove the young leaves from the tip of male and female gametophytes, using a needle scrape the tip of the gametophyte into drops of water on slides to remove the sex organs. Add a coverglass and examine each microscopically. The male organ or antheridium has stalk cells and jacket cells that surround the sperm (male gametes). The female organ or archegonium has stalk cells and venter cells that enclose one large non-motile egg (female gamete). What is the function of the antheridial jacket cells and archegonial venter cells? The female gamete or egg remains in the archegonium on the tip of the gametophyte.

While the gametophytes are flooded the sperm swim from the antheridium to the archegonium and down the canal of the archegonium. The fusion of egg and sperm occurs in the venter of the archegonium. The process of the egg and sperm fusing is called fertilization. The one cell which results from the fusion of a sperm with an egg is called a zygote. Examine the demonstrations of longitudinal sections of antheridia and archegonia.

B. SPOROPHYTE

The mature sporophyte in mosses resembles a small golf club 2-12cm long. Dissect to find the following: a foot which is embedded in the top of the gametophyte and secures the sporophyte to the gametophyte; a stalk which elevates the capsule or spore-producing sporangium (spore case) for better spore dispersal. A mature capsule possesses a lid called an operculum. The rim of the capsule under the operculum possesses many teeth. The teeth are hygroscopic structures because they flex in and out of the capsule in response to humidity changes. Under the dissecting microscope remove the operculum, dry it, and then note the response of the teeth as you breathe on them. Would the hygroscopic nature of the teeth aid in spore dispersal? How?

Fig. 18.7
Mosses, life-sized.
A: Male gametophyte.
B: Female gametophyte.
C: Female gametophyte of
a smaller species of
moss, with a mature
sporophyte growing
from its tip.



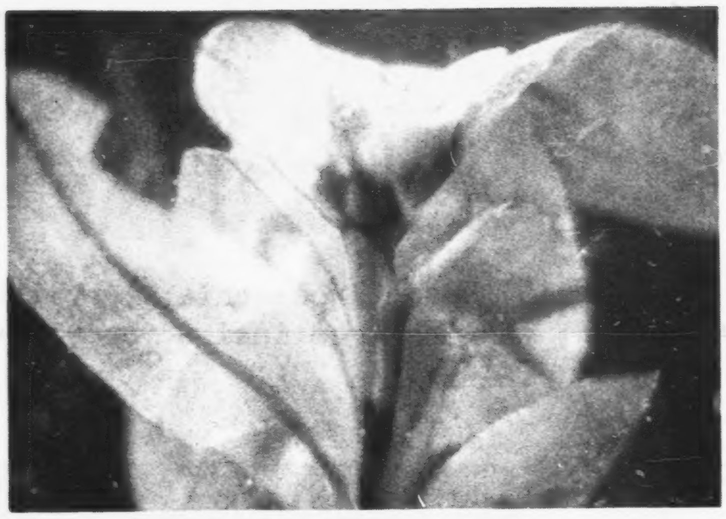


Fig. 18.8 Moss, tip of male gametophyte, magnified x20.

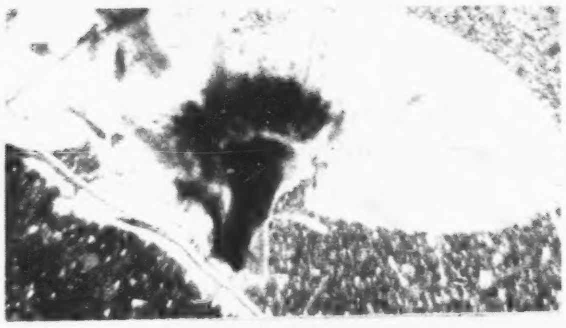


Fig. 18.9 Moss, crushed tip of male gametophyte, magnified x20.

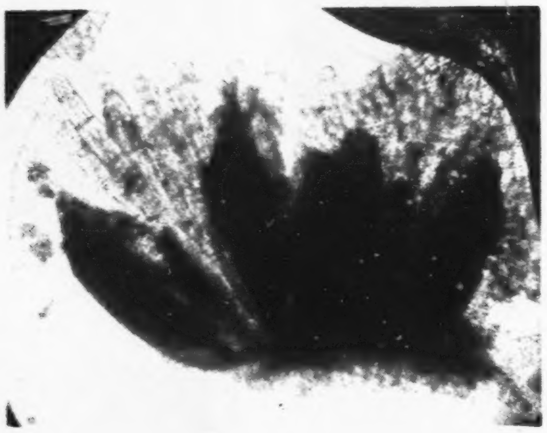


Fig. 18.10 Moss, crushed male gametophyte, showing antheridia (dark, ovoid) and sterile hairs. Magnified x100.

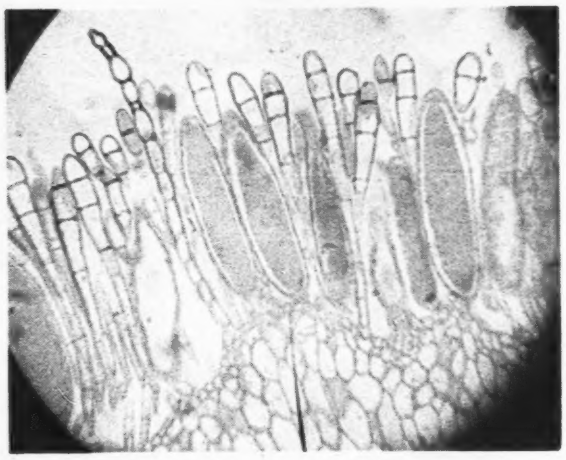


Fig. 18.11 Moss antheridia, longitudinal section, showing sperm inside.
Magnified x100.

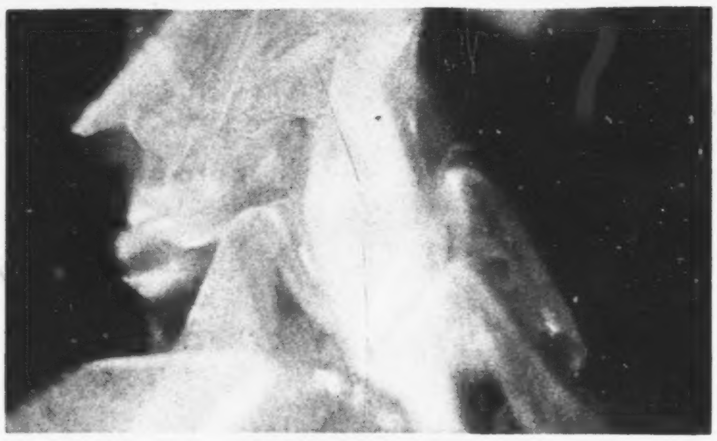


Fig. 18.12 Moss, tip of female gametophyte, magnified x20.

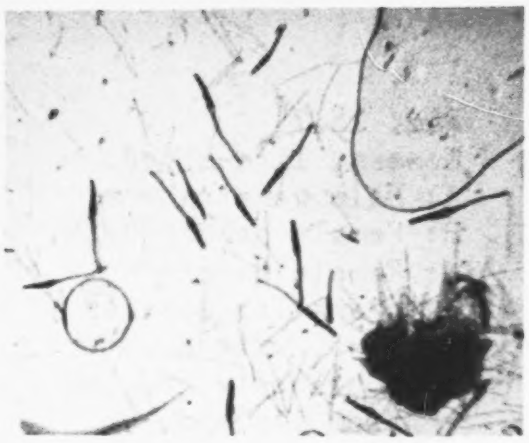


Fig. 18.13 Moss, crushed tip of female gametophyte, magnified x20.

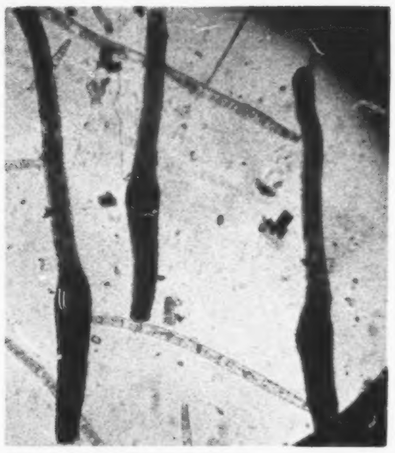


Fig. 18.14 Moss, crushed female gametophyte, showing archegonia and sterile hairs, magnified x100.

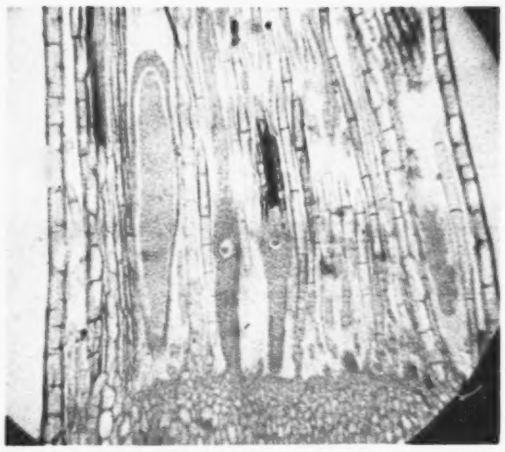


Fig. 18.15 Moss, archegonia, longitudinal section, showing eggs inside venter cells, magnified x100.

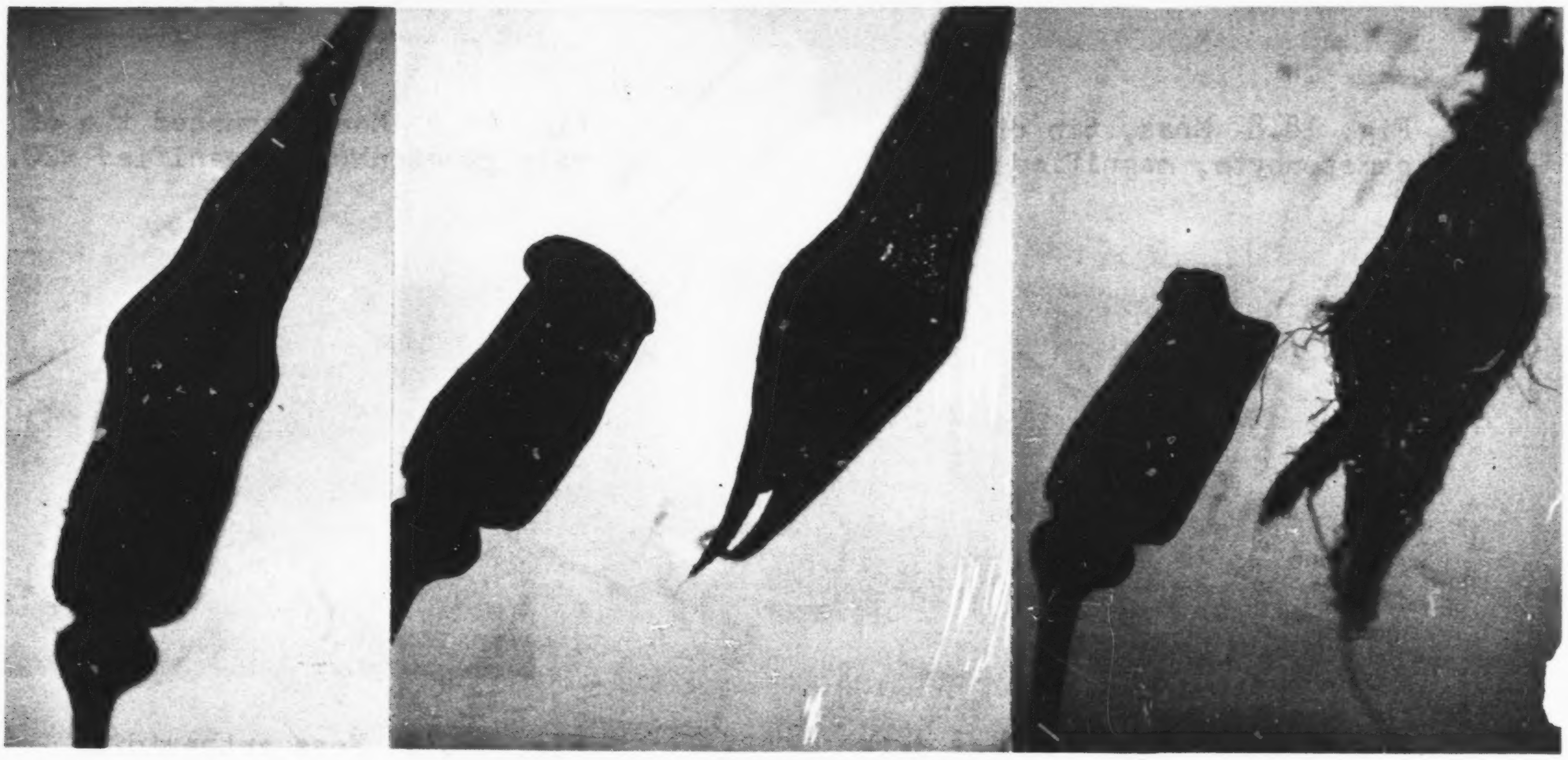


Fig. 18.16 Moss sporophyte capsule, whole, then dissected, then dried. Magnified x20.

IV. PHYLUM TRACHEOPHYTA

In the Tracheophytes, it is the sporophyte that is the dominant plant in the alternation of generations. This is opposite from the situation in the Bryophytes. In Tracheophytes, the sporophyte is independent at maturity while the gametophyte is either independent at maturity (ferns) or dependent on the sporophyte (seed plants). The tracheophyte's sporophyte has two conducting or vascular tissues: The xylem which conducts water and minerals and the phloem which conducts dissolved food. The sporophyte consists of three vegetative organs; root, stem and leaves.

A. FILICINEAE - FERNS

The fern sporophyte is the plant one sees growing as a house plant and in public buildings. When young, the fern sporophyte is dependent on the gametophyte but as it matures it becomes completely independent.

1. Gametophyte

You will have observed the growth of the fern gametophyte from the spores you sowed on agar, but you will probably not have had time to see the development of the antheridia and archegonia. Demonstration slides are provided showing these structures.

As in the mosses, a flagellated sperm will swim from an antheridium on one gametophyte to the neck of an archegonium on another gametophyte, down the canal and unite with the egg. What is the name given to the process in which a sperm units with an egg?

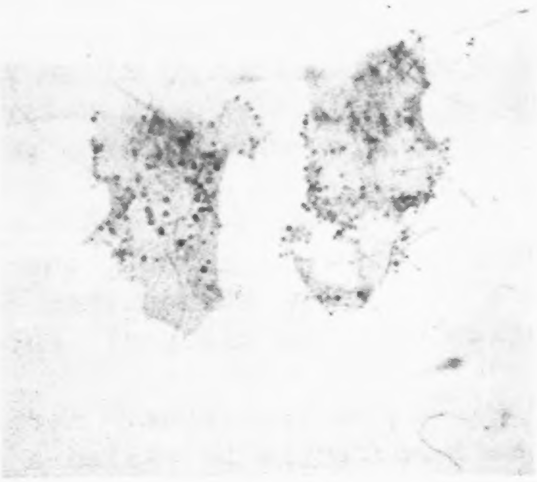


Fig. 18.17
Two young fern gametophytes, each dotted with antheridia, magnified x30.

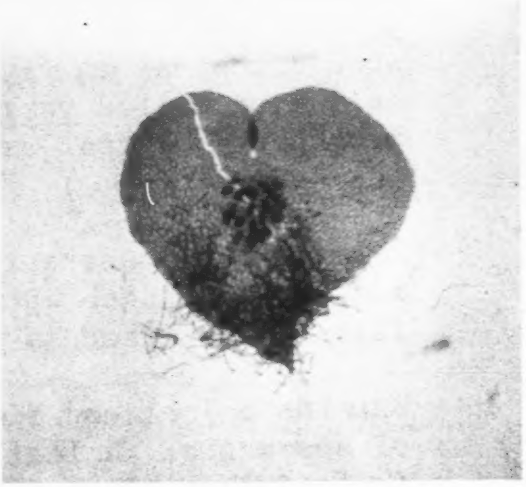


Fig. 18.18
An older fern gametophyte, with the necks of archegonia showing, magnified x30.

2. Young Sporophyte (Fig. 18.19)

The zygote will develop into an embryo in the venter of the archegonium. Examine the demonstration slide showing the embryo attached to the gametophyte and identify the young multicellular embryo: a foot which serves as an absorbing and anchoring organ for the young sporophyte as it is attached to the gametophyte; a primary root which begins absorbing water and minerals for the young sporophyte from the soil; a primary leaf which is the first photosynthetic organ of the young sporophyte and a stem which continues to grow and form larger and larger leaves at its end.

As the stem of the embryo developes, the gametophyte and the foot, primary root and primary leaf of the embryo disintegrate. What part of the embryo does the mature sporophyte develop from? What is meant by the statement, "The new fern sporophyte must grow where the gametophyte does?"

3. Older Sporophyte

Examine a fern sporophyte (see Fig. 18.20) and note the large leaves consisting of a blade and petiole. The young leaves appear as tight coils and as they mature unroll and straighten up. Many ferns have divided leaves; that is, many leaflets are attached to the petiole.

Ferns in our area normally do not have stems above ground, but instead have a perennial underground stem called a rhizome (see Fig. 18.21). Examine the rhizome demonstration, and note the many roots arising from it.

Examine a demonstration slide of cross section of the rhizome (stem), and note the clusters of xylem cells with phloem cells around them as in fig. 18.22. These clusters of xylem and phloem cells are called vascular bundles. Each of the vascular bundles around the central axis branches into a leaft at some point on the stem.

Examine a demonstration of cross section of a root, and note the many xylem ridges with phloem alternating between them as in fig. 18.23. What is the function of xylem tissues? Phloem tissues?

Examine a leaf and find the sori on the back side. Sori are clusters of sporangia. A leaf which bears sporangia is called a sporophyll (a leaf that bears spores). Not all leaves of ferns will bear spores and florists often remove the sporophylls from plants because customers believe the sori are caused by a disease.

4. Specialization of Sporophylls in Ferns

In order that you understand the concept of a sporophyll as a leaf which bears sporangia, a series of demonstrations is set up showing an increasing specialization of the sporophylls.(Figures 18.24 - 18.26). Note that the series begins with leaves that are

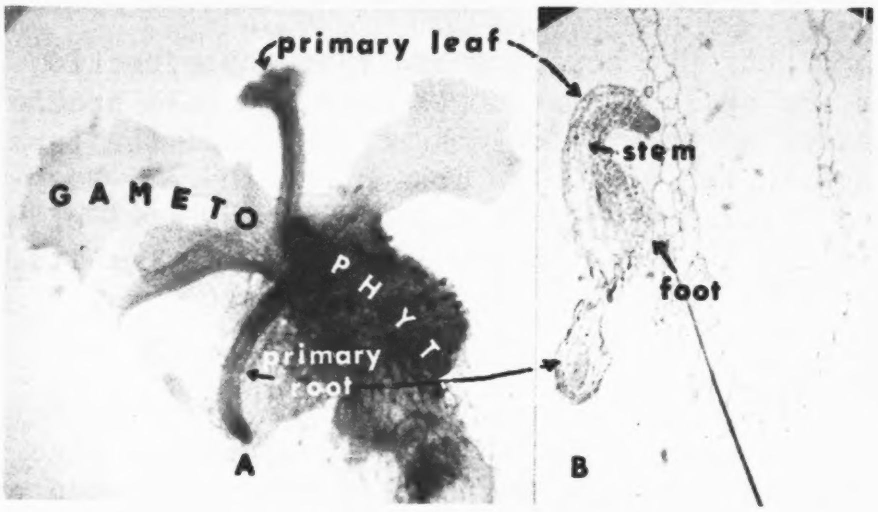

primary leaf
stem
GAMETO
PHYT
foot
primary root
A
B

Fig. 18.19 Fern, young sporophyte growing from female gametophyte. A, whole mount x30. B, longitudinal section x100 of young sporophyte and gametophyte.

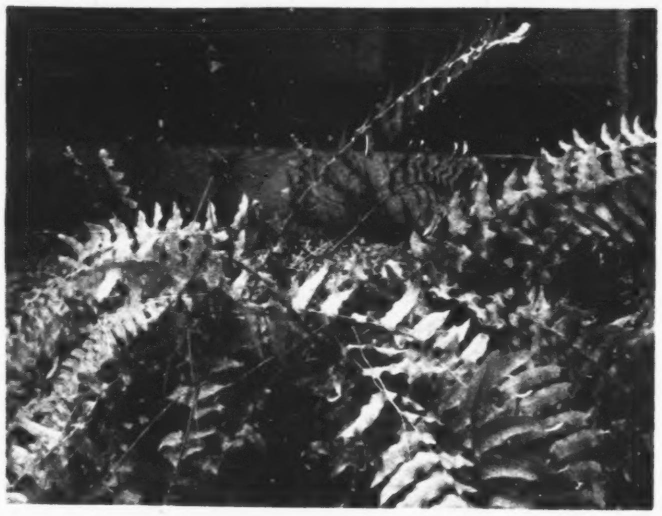


Fig. 18.20 Fern, older sporophyte. Each leaf grows from the ground.

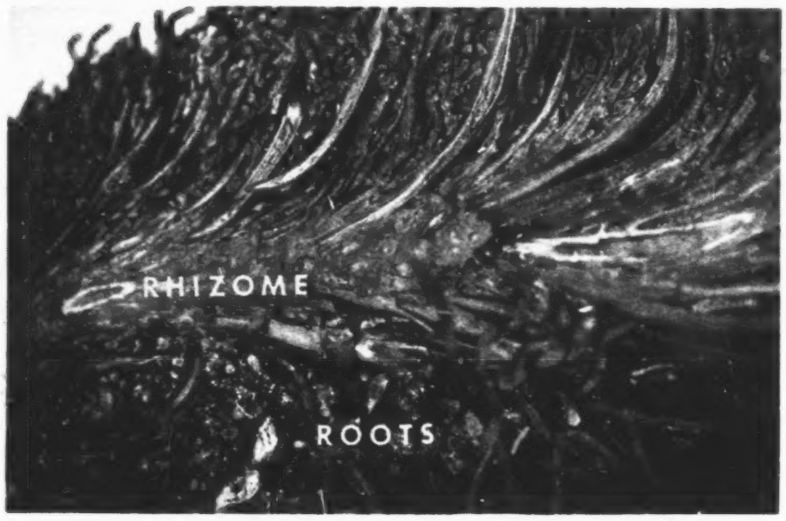

RHIZOME
ROOTS

Fig. 18.21 Part of a fern rhizome with roots attached; longitudinally sectioned, life sized.

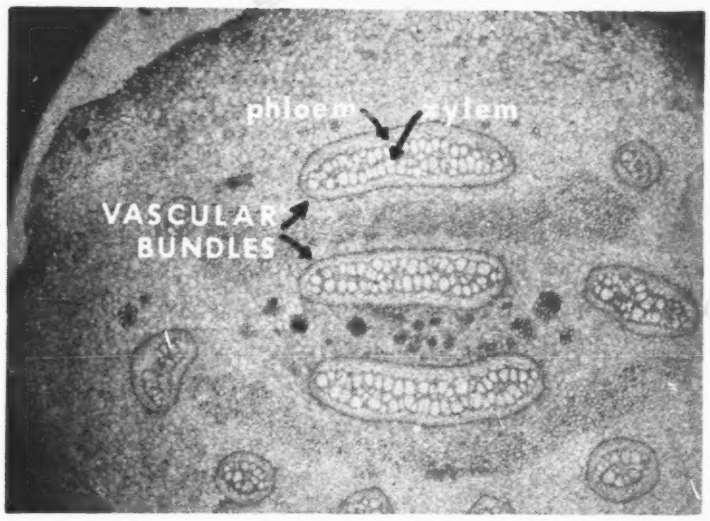

phloem
xylem
VASCULAR BUNDLES

Fig. 18.22 Cross section of fern rhizome, showing vascular bundles, x30.

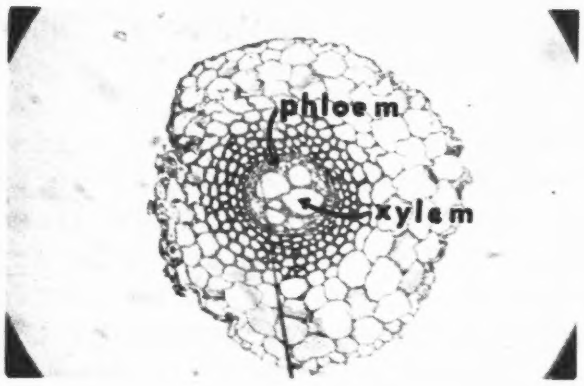

phloem
xylem

Fig. 18.23 Cross section through fern root, x100.

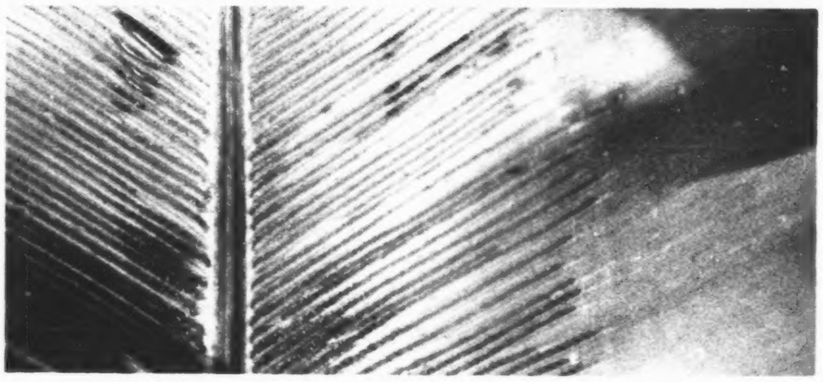


Fig. 18.24 Back of leaf of Bird's Nest Fern, showing sori. In this fern, each leaf is both spore-producing and photosynthesizing. Compare with 18.24 & 18.25.

both sporebearing and photosynthesizing and that these functions become separated as the sporophyll becomes more and more specialized, for spore production only. How does the size of the fern gametophyte compare with the size of a moss gametophyte? How does the size of the fern sporophyte compare with the size of a moss sporophyte? Is the fern sporophyte more dependent on its gametophyte than the moss sporophyte is on its gametophyte?

B. GYMNOSPERMAE -- CONIFERS, EVERGREENS

The sporophyte plant in gymnosperms is usually a tree, such as pine or spruce. The spores that the sporophyte produces are never shed at all, but remain on the sporophyll and develop between the leaves of cones. A cone is a cluster of sporophylls.

1. Sporophyte

a. Leaves

Examine the needle-like leaves of the pine branch on demonstration. Each needle is a leaf. Examine a demonstration slide showing a cross section through a leaf, and refer to fig. 18.27 to find the parts. Note the vascular bundle containing zylem and phloem, the resin canals lined with resin-secreting cells, the epidermis and stomata (openings for gas exchange) and cortex cells containing chloroplasts. In what basic way does a leaf of the pine sporophyte differ from a "leaf" of the moss gametophyte?

b. Stems

Examine a cross section demonstration slide (Fig. 18.28) of a pine stem and note the annual rings. How old is the stem section? The annual rings are actually constructed of secondary xylem (wood) cells which have been formed by the vascular cambium. The rings are formed due to the difference in size of the cells produced by the vascular cambium in the spring (spring wood) from the size of the cells produced in the summer (summer wood). The region of the vascular cambium is between the wood and the bark. The inner part of the bark is made up of cells of the secondary phloem and the outer part is made up of cork cambium and cork cells. What are the functions of the xylem and phloem tissues?

Microscopically (Fig. 18.29) examine a demonstration slide showing three views of pine wood: cross (transverse) section, radial section, and tangential section. In the three views note the appearance of tracheids (with pits), rays and resin canals. Why are there no phloem cells seen in these sections? Study the blocks of wood on demonstration and determine the various views mentioned above.

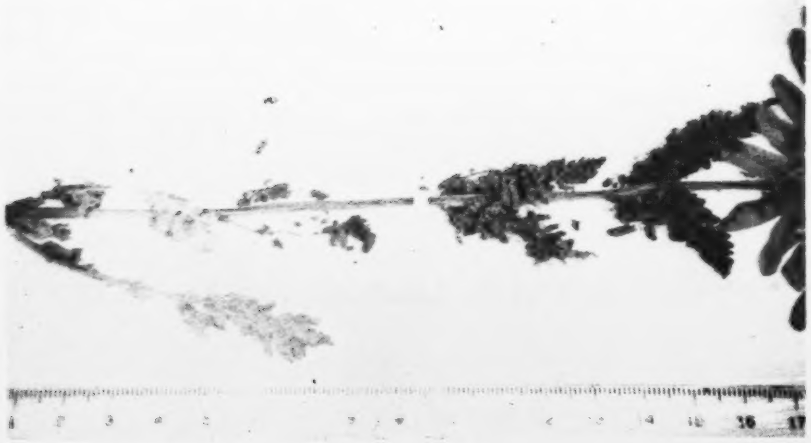


Fig. 18.25 Part of Interrupted Fern. Some leaflets in the middle produce spores.

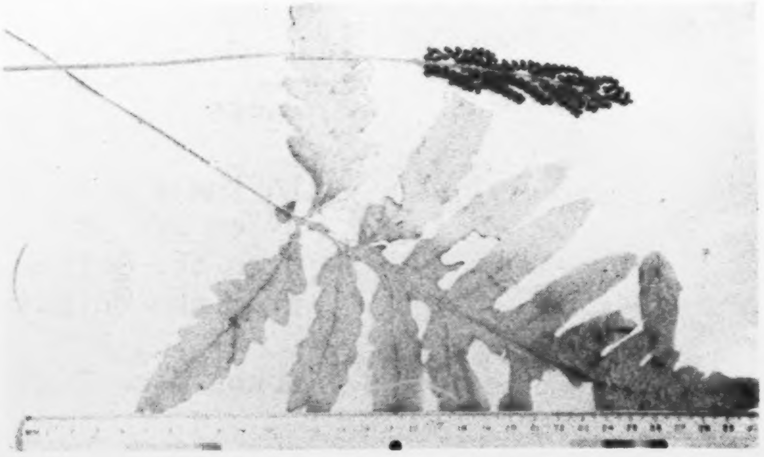


Fig. 18.26 Two leaves of Sensitive Fern, one entirely spore-producing.

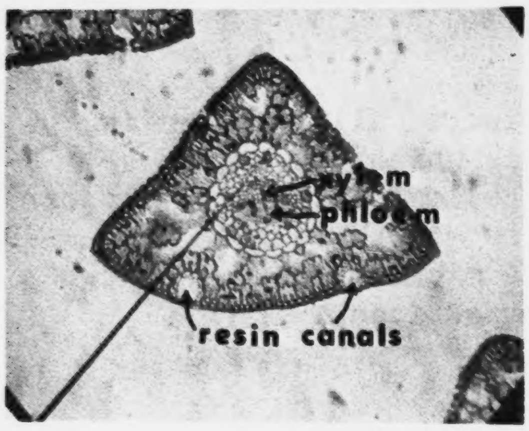

xylem
phloem
resin canals

Fig. 18.27 Cross-section of Pinus leaf x100.

Fig. 18.28 Cross-section of Pinus stem, three years old.

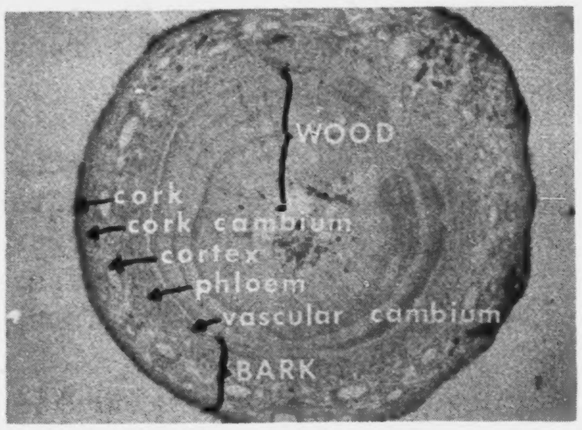
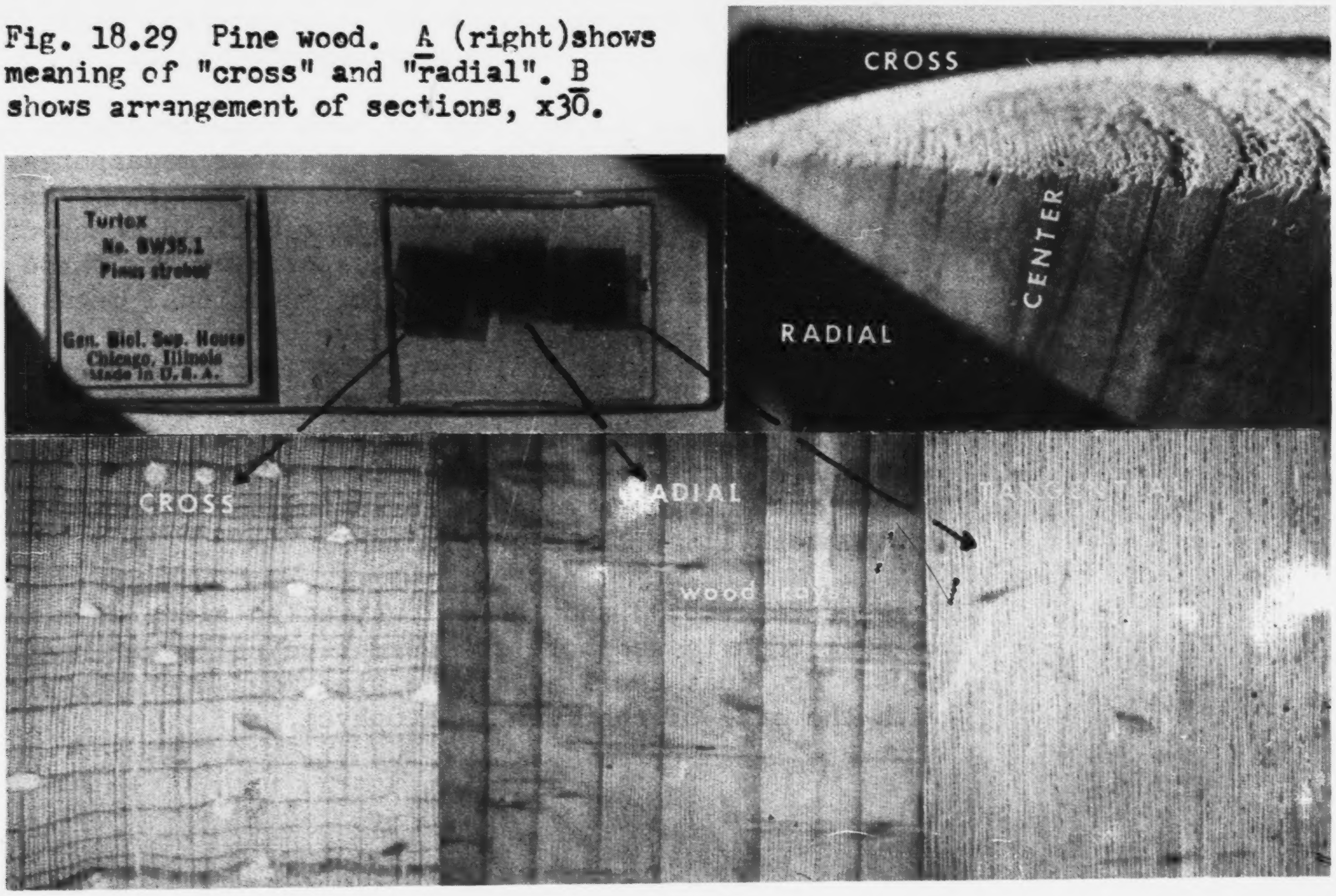

WOOD
cork
cork cambium
cortex
phloem
vascular cambium
BARK

Fig. 18.29 Pine wood. A (right) shows meaning of "cross" and "radial". B shows arrangement of sections, x30.


CROSS
CENTER
RADIAL
Turtox
No. BW35.1
Pinus strobus
Gen. Biol. Sup. House
Chicago, Illinois
Made in U.S.A.
CROSS
RADIAL
wood rays
TANGENTIAL

c. Roots

In the slides of young pine roots (Fig. 18.30) locate the xylem and phloem tissue. The xylem is centrally located (red) with the phloem external to it. How do roots of pine differ from the rhizoids of the moss and fern gametophytes?

d. Cones (see Fig. 18.31)

The megastrobilus (female cone) is the one which persists for some time and is frequently used in decorations of various kinds. Each scale of the megastrobilus is a megasporophyll. There are two ovules per megasporophyll. Each ovule consists of a megaspore mother cell, within a megasporangium, within the integuments.

The microstrobilus (male cone) in pine usually appears in clusters and are temporary structures which dry up and disintegrate after producing their pollen. Examine a cluster of microstrobili at the base of a new growth of stem and leaves. Each microsporophyll of the microstrobilus has two microsporangia attached to its lower surface.

2. Gametophyte

a. Megagametophyte

Study fig. 18.32 and the demonstration slide showing a young ovule and note the megasporphyll, integements, megasporanium with the megaspore mother cell contained in it.

The one megaspore mother cell in each megasporangium undergoes the process of meiosis and forms four megaspores. The one megaspore mother cell in each megasporangium undergoes the process of meiosis and forms four haploid megaspores. These are retained within the megasporanium but three of the four megaspores disintegrates. The functional megaspore which remains divides many times, and developes into a (haploid) megagametophyte which will bear only female gametes. The mature pine megagametophyte consists of many non-green nutritive cells and two to four archegonia. Each archegonium contains a single egg. The mature megagametophyte remains inside the integumented megasporangium and attached to the megasporophyll of the megastrobilus.

Examine Fig. 18.33 and the demonstration slide of a mature megagametophyte and note the nutritive cells, archegonia and eggs. How does the size of the pine megagametophyte compare with the size of the fern gametophyte?

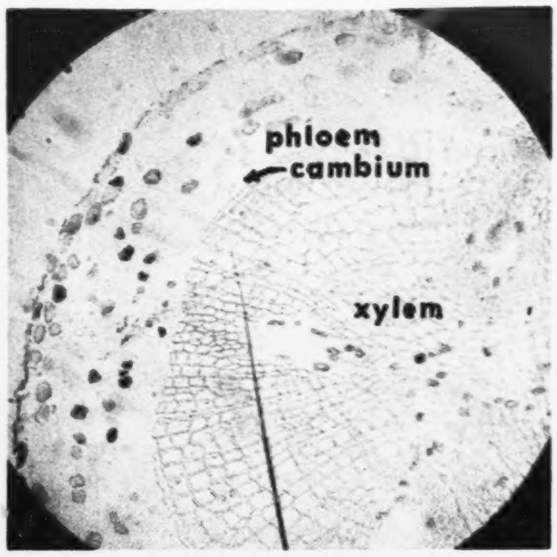

phloem
cambium
xylem

Fig. 18-30 Pine root, cross section, x100.

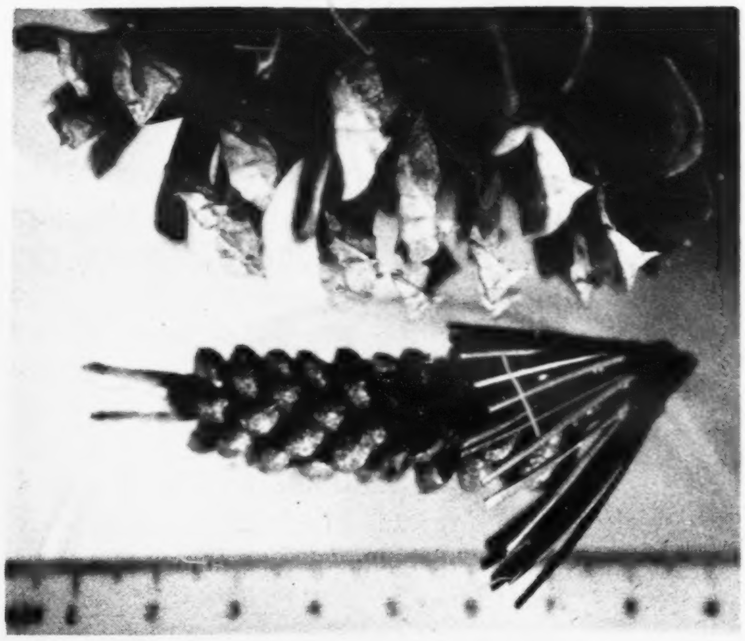


Fig. 18.31 Pine strobili, life sized.
Above: one megastrobilus.
Below: a group of many microstrobili.

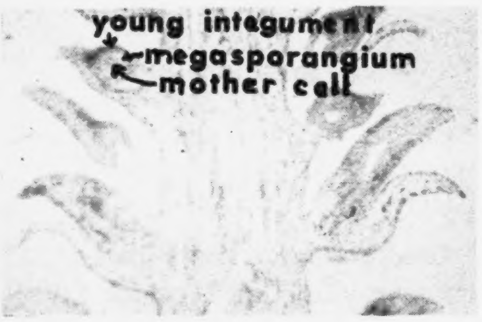

young integument
megasporangium
mother cell

Fig. 18.32 Longitudinal section of a young megastrobilus, x30.

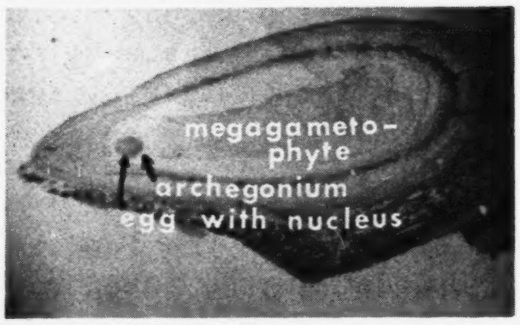

megagametophyte
archegonium
egg with nucleus

Fig. 18.33 Section of mature megasporophyll with megagametophyte, x30

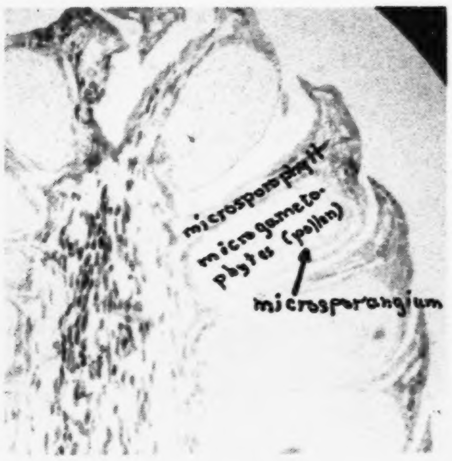

microsporophyll
microgametophytes (pollen)
microsporangium

Fig. 18.34 Longitudinal section of mature microstrobilus, x100.

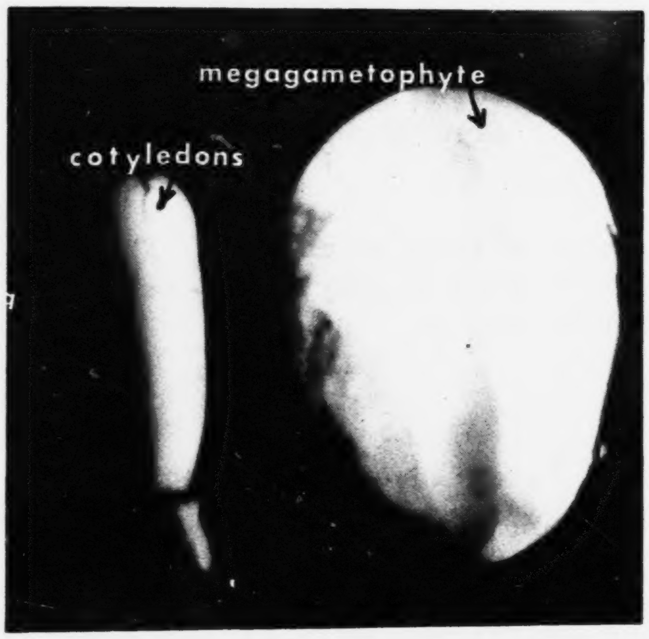

megagametophyte
cotyledons

Fig. 18.36 Dissected pine seed, embryo dissected out, x30.

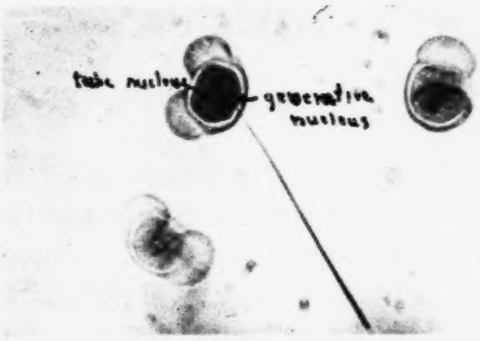

tube nucleus
generative nucleus

Fig. 18.35 Pine pollen x430.

b. Microgametophyte

Examine Fig. 18.34 and a slide showing a longitudinal section through microstrobilus and note the microsporophylls and microsporangia. A microsporangium contains many microspore mother cells. Each microspore mother cell undergoes meiosis to produce four microspores. Each microspore produced has the potential to develop into a young microgametophyte (pollen grain) which will bear only male gametes.

A microgametophyte is ready to be released from the microsporangium when it possess four cells: 2 nonfunctional gametophyte cells, a generative cell and a tube cell. Examine Fig. 18.35 and the demonstration slide of a young microgametophyte (pollen grain) and note the tube cell and generative cell. The prothallial cells have probably already degenerated. The ear-like structures on the pollen grains are extensions of the microspore wall which add buoyancy and aid in wind dissemination.

The transfer of pollen (young microgametophytes) from the microstrobilus to the megastrobilus is termed pollination. In pines pollination is accomplished mainly by the wind.

c. Fertilization

The young microgametophytes land on the female cone and pass through an opening in the integuments (micropyle) and come in contact with the megasporangium. The tube cell of the microgametophyte then proceeds to grow through the megasporangium. As the tube cell approaches the megagametophyte the generative cell of the microgametophyte divides once to produce two non motile sperm. One of the sperm cells of the mature microgametophyte disintegrates while the other units with the egg in the process of fertilization to form a zygote.

Is water a necessary medium for fertilization to occur in pine? How has the pine freed itself from the necessity of water for fertilization?

The zygote then divides many times to form an embryo. The embryo and nutritive cells of the megagametophyte are retained within the megasporangium and integuments.

3. Young Sporophyte (Fig. 18.36)

Dissect a pine seed and note the hard outer layer or the seed coat which has developed from the integuments surrounding the megasporangium. Remove the seed coat carefully and note a brown papery layer, the megasporangium. To the inside of the megasporangium is the mass of fleshy nutritive cells of the megagametophyte. Remove these fleshy cells carefully and note a cigar shaped structure, the embryo. The embryo will have a number of cotyledons or seed leaves at one end and a hypocotyl or potential stem and root system at the other end. How many cotyledons are there?

At the base of, and enclosed by, the cotyledons there is an epicotyl which will give rise to the stem and leaves when the seed germinates. How do the seed coat, nutritive tissue and embryo function for the success of the seed? How does the wing on the seed, which is an extension of the seed coat, function? It is interesting to note that the pine seed consists of three generations: the parent sporophyte generation in the seed coat and megasporangium, the megagametophyte generation in the nutritive tissue, and the new sporophyte in the embryo. Do mosses have seeds? How are seeds advantageous to plants which produce them?

Directions will be given in the lecture for the planting and germination of a pine seed. Keep accurate records of the germination of these seeds, in order to trace the development of the following: cotyledons, stem and root portion from the embryonic hypocotyl and stem, and leaf portions from the embryonic epicotyl, the nutritive tissue in the seed, and the seed coat. What is the function of the cotyledons in pine?

Is the pine gametophyte parasitic on the pine sporophyte? How does this situation compare with the gametophyte-sporophyte relationship in the moss ?

LABORATORY WEEK 19:
ANGIOSPERMAE

I. BACKGROUND

The Angiosperms include all of the flowering plants; such as elm, lilac, rose, corn, orchid and palm. The angiosperm sporophyte is the plant which bears the flowers. The flower in angiosperms resembles the gymnosperm cone in many ways, but the seeds in angiosperms are enclosed inside megasporophyll tissue whereas the gymnosperm seeds lack a covering and are borne on the surface of the megasporophyll. (Gymno - = naked, Angio - = hidden, -sperm = seed)

II. CULTURE OF BUCKWHEAT AND PINE SEEDS

This work must be carried out several weeks in advance, and some observation will be necessary before the nineteenth week. Proceed as follows:

1. Write your name and the date on a paper cup.
2. Fill the cup with soil to within 2 cm. of the top.
3. Place a pine seed and 3 or 4 buckwheat seeds on the surface of the soil equidistant from each other.
4. Using your finger push the seeds down about ½ cm. below the surface. Cover the seeds if necessary by adding a little more soil.
5. Water carefully so the soil is damp (not soggy).
6. Wrap the entire assembly in a plastic bag and place it in the window.
7. Keep your plants watered and if mold beings to develop, take the bag off for a few hours. When the plants begin to grow, it will be necessary to support the bag or leave it off entirely. If the latter is necessary, you must water the plants very frequently to avoid drying out.
8. Keep a good record of the developmental sequence of the plants, by means of dated diagrams. You will use the sequence and the plants in the previous work on Pines and in the work that follows.

III. SECTIONING AND STAINING PLANT PARTS

You should begin this work about a week before the ninteenth week, so you will have permanent slides of your own with which to work. Carefully uproot one of your buckwheat plants and place it in water so it will not dry out. You will be making freehand sections through a leaf, the stem and the root, and staining them. Detailed directions are given for only the leaf, but be sure to repeat them for the stem and root.

1. Put about 10 ml. of water in a test tube and keep it ready to receive the sections.
2. Remove one leaf from the buckwheat plant.
3. Cut a piece of dry pith into two halves, and then mount the leaf in it like a sandwich.

4. Using a sharp razor blade cut thin slices of pith and leaf. Place these sections immediately into water in the test tube.

5. When ready to stain (after all the sections have been cut), pour off the water, add 50% ethanol and leave it for at least one hour. Overnight is not too long.

6. Pour off the ethanol, and with a dropper add about 1 ml. of 1% safranin. Let stand 1 hour or more. Overnight is usually not too long.

7. Wash well in water using 2 or 3 changes.

8. 50% alcohol for 2 - 5 minutes.

9. Destain in 70% acid alcohol. A brief rinse is all that is necessary.

10. Wash well through several changes of 50% ethanol.

11. Add 1 ml. of fast green counterstain. The time in this stain will vary according to the thickness of the sections. Start with one minute and if this time is not correct, adjust. Check, by examining the sections microscopically in 50% ethanol. Mount to see if any of the cells have a definite green color. If not, repeat the stain.

12. Rinse (briefly) with 95% alcohol until the stain ceases to come out of the sections freely.

13. Two changes of ethanol - xylol, 5 - 30 minutes each.

14. Xylol. Store sections in xylol and study your sections in xylol on slides with cover slips. Do not allow the sections to dry out at any time during the staining procedure or while you are studying them.

15. The slides may be made permanent by placing the stained section in a drop of H.S. Resin and adding a cover slip. The slides must be kept flat untilthe Resin has dried around the edges of the cover slip.

16. The correct contrast of colors are: red nuclei, green cytoplasm, pink to red chloroplasts and green or red cell walls.

17. Do all the above with leaf, stem and root sections.

IV. SPOROPHYTE

Use your buckwheat plants and other plants, plus your prepared slides and other prepared slides, to find the following:

A. Leaves

1. Parts and Arrangement

Angiosperm leaves consist of two general parts: a broad blade, and a narrow stalk or petiole which attaches the leaf to the stem. There is always a bud in the axil of a leaf (that is, in the angle which the petiole makes with the stem). This bud is called an axillary bud and will develop either into a branch stem bearing either leaves or flowers or both. Examine the leaves of the buckwheat plants and note the blade and petiole. The axillary buds on the buckwheat are not easily seen because in house-grown plants they do not remain as buds but develop into stems as soon as they are formed. Are the leaves arranged in a definite way on the stem?

2. Microscopic examination

Compare your prepared leaf section with a professionally prepared demonstration slide of a cross section through a lilac leaf as in Fig. 19.1. Note the cuticle, a waxy layer of cutin secreted by the upper epidermal cells. What is the function of the cuticle? Examine the upper epidermis cells. Do any of the cells contain chloroplasts? Note the stomata with their guard cells. What is the function of a stoma? Do you know how the pore size is regulated?

The layer of elongate cells "hanging" from the upper epidermis is called a palisade layer. These cells contain many chloroplasts and are the main tissue of the leaf which carries on photosynthesis. The large irregular cells below the palisade layer make up the spongy mesophyll and function primarily in water and food storage but there is some photosynthetic activity in these cells. Below the spongy mesophyll is the lower epidermis. Are there stomata in in the lower epidermis?

Note the cross section of veins on your slide and determine the xylem (red) tissue and phloem (green) tissue in these veins. Is the xylem always above or below the phloem tissue? How do you account for this constant arrangement of vascular tissue in the leaf?

The leaf structure you have just studied is common for leaves of plants which obtain an average amount of water such as maple, oak and cultivated trees and shrubs. These plants because of their water needs, are called mesophytes. Plants which grow in very dry climates (xerophytes) and those which grow in water (hydrophytes) have an entirely different structure from that you have just studied. What is the main function of a leaf? Are angiosperm leaves part of the sporophyte generation or gametophyte generation?

B. Stems

Angiosperm stems grow from the tip out, due to the activity of a group of embryonic cells in the stem apex called the apical meristem. The apical meristem is usually protected by many young overlapping leaves.

1. Dissect a young stem tip from your buckwheat plant under a dissecting microscope by removing the young overlapping leaves. How are these leaves arranged on the stem?

2. Obtain a prepared microscopic slide of longitudinal section through a Coleus stem tip as in Fig. 19.2. Note the apical meristem at the very apex of the section just under the epidermis. The young cells which are left behind the apical meristem elongate and mature into various tissues such as xylem and phloem. You will see young vascular tissue as dark interrupted strands running longitudinally in the section. The outgrowths on the stem tip are young leaves. These leaves originate when groups of embryonic cells of the stem apex are left behind and form the nucleus of a new leaf. Note in the axil of the young leaves the axillary buds. What will these axillary buds develop into?

3. Compare your buckwheat stem section with a demonstration of a sunfloweras in Fig. 19.3. In the stem cross-section find the large thin-walled parenchyma cells in the center of the stem making up the pith; the ring of vascular bundles each containing xylem to the inside and phloem to the outside; the cortical parenchyma cells containing chloroplasts located between the vascular bundles and the epidermis. What is the function of the cortical parenchyma? Can you see any stomata in the epidermis? All of the tissues in the young stem are primary tissues because they have arisen as a result of the maturing of cells left behind by the apical meristem. Explain how the stem increases in length as a result of primary tissue maturations.

4. Secondary growth or increase in girth is accomplished by the vascular cambium (a lateral meristem) which originates between the xylem and phloem in the young stem. The vascular cambium divides and forms cells to either side of it. The cells which develop to the inside are called secondary xylem cells and the cells which develop to the outside are called secondary phloem cells. As the stem increases in girth due to the vascular cambium's producing cells, splits occur in the epidermis. A cork cambium forms in the cortical parenchyma and forms cork cells to the outside. As the stem continutes to increase in girth the epidermis no longer fits and it splits open. The cork takes its place. In this way the cells in the stem are prevented from dying out. Explain how a plant stem increases in girth.

   Study the demonstration cross section of a basswood stem. Note the annual rings of secondary xylem, vascular cambium region, secondary phloem, cork cambium region and cork. Where would you cut a tree to determine its approximate age by counting annual rings?

C. Roots

Angiosperm roots increase in length at the tips by means of an apical meristem in a manner similar to the stem tip.

1. Obtain some buckwheat roots by digging them up and gently washing them free of soil. Keep the roots in water at all times. Mount 1 or 2 of the smaller roots in a drop of water on a slide and note the root cap, a group of cells which protects the apical meristem as it moves through the soil. Note also the numerous root hairs

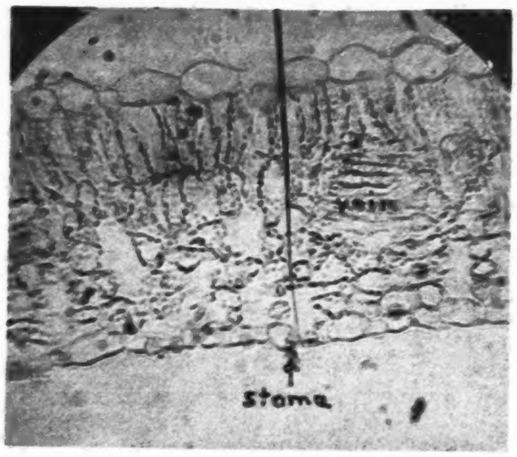


Fig. 19.1 Syringa leaf, cross section, magnified x430.

Fig. 19.2 Coleus stem tip, longitudinal section, magnified x30.

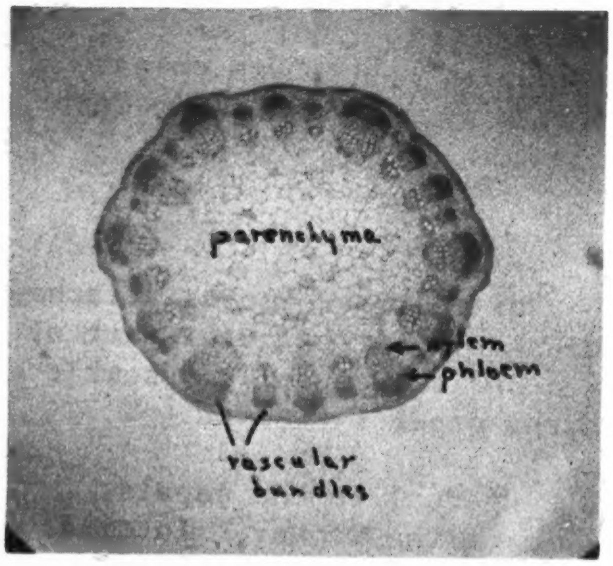

parenchyma
xylem
phloem
vascular bundles

Fig. 19.3 Sunflower stem, cross-section, magnified x100.

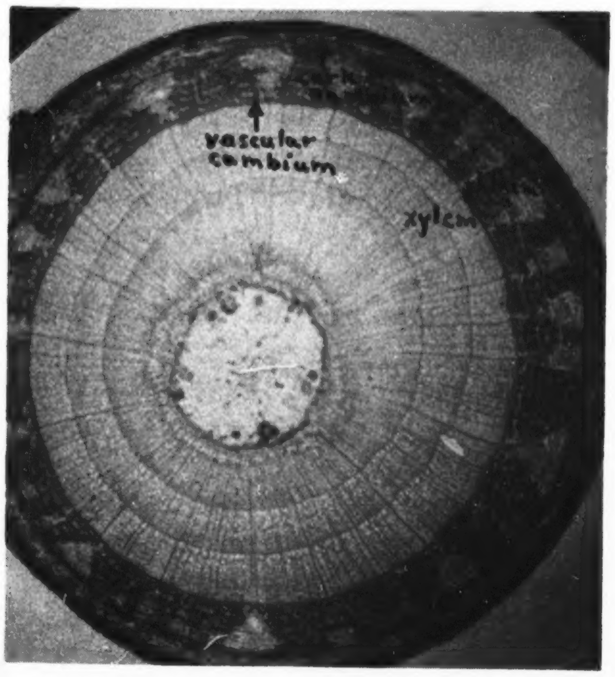

vascular cambium
xylem

Fig. 19.4 Three-year basswood stem, cross section, x30.

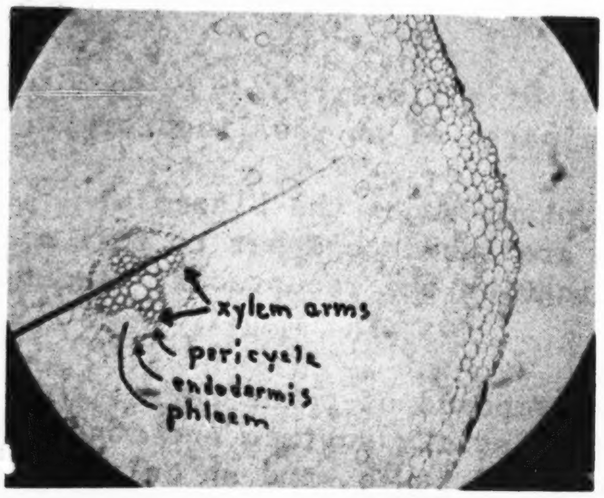

xylem arms
pericycle
endodermis
phloem

Fig. 19.5 Buttercup mature root, cross-section, magnified x100.

which are one cell epidermal structures. Root hairs are the main water absorbing structures of the plant.

2. Compare your stained cross section of a buckwheat root with a prepared butter-cup root section as in Fig. 19.5. Note the "xylem arms", the pericycle (thin-walled cells which form a ring around the xylem and phloem), the endodermis (a layer of cells just outside the pericycle), the cortical parenchyma which contains numerous starch grains and finally, the epidermis. What is the function of each of the root tissues mentioned? Why are all of the tissues described called primary tissues? The pericycle is the region of the root which gives rise to branch or secondary roots.

3. Secondary tissues are formed in the root in a manner similar to the stem. Study a cross section of an older root such as the carrot. Can you distinguish cork, cork cambium, secondary phloem, vascular cambium, and secondary xylem? The annual rings in root secondary xylem are not as pronounced as in the stem. How do you account for this?

Review Question: How does the size of the angiosperm sporophyte compare with the size of the sporophytes found in moss, in fern and in gymnosperms?

D. Flower

The angiosperm flower is basically a number of modified leaves attached to a stem (receptacle). The calyx made of sepals is the outer part of the flower, and its parts are usually green and structurally resemble the leaves of the plant more closely than the other floral parts.

The corolla made of petals is located just internal to the sepals. The petals are often the most colorful parts of the flower. Internal to the petals are the stamens (microsporophylls) which eventually produce the pollen (microgametophytes). The innermost floral parts are the pistils (megasporophylls and carpels) which eventually produce the ovules (megagametophytes). Many plants have only one pistil, which may be made of one single carpel or several fused carpels. Many flowers lack one or more of the structures mentioned above, and still other flowers will have these structures fused to one another in a variety of ways.

1. Look at the demonstration of a model of a flower, to see the arrangement of the above parts of an idealized organ.

2. Look at a flower of your buckwheat plant under the binocular microscope. The sepals and petals are fused together to form a structure called a perianth. Distinguish the receptacle, stamens and pistil.

   a. The pistil (megasporophyll) is composed of three parts: the top or stigma which consists of a sticky surface and functions to receive the pollen, the elongated style, and an enlarged base, the ovary, which contains the seeds.

Each seed has developed from a megasporangium and its integument layers. The one functional megaspore mother cell in a megasporangium divides by meiosis and produces four megaspores (see V. A below).

b. The stamen (microsporophyll) is composed of an elongate filament and an enlarged terminal anther. The anther contains four elongate microsporangia, each containing numerous microspore mother cells. Each microspore mother cell undergoes meiosis to produce four functional microspores (see V.B below).

V. GAMETOPHYTE

Demonstration slides showing various stages of gametophyte development have been set up in the laboratory. You should understand the general process of gametophyte development.

A. Megagametophyte

The one functional megaspore mother cell in the megasporangium divides by meiosis and produces four megaspores. Three of the megaspores disintegrate and the one functional megaspore divides a number of times (how many?) to produce an eight-nucleate cell. Cell walls then develop around the nuclei resulting in a seven-celled megagametophyte with six of the cells having one nucleus each and one of the cells having two nuclei.

One of the six cells becomes the female gamete or egg, which is not enclosed in an archegonium. The two nuclei in the binucleate cell are called the polar nuclei. Where is this seven-celled mature megagametophyte located?

B. Microgametophyte

Each microspore cell divides to produce a two celled microgametophyte (pollen grain). The two cells of the pollen grain are the generative cell and the tube cell. Examine the prepared slide of pollen and distinguish the spindle shaped generative cell and the tube cell which occupies most of the pollen grains. Why does a plant species produce much more pollen (young microgametophytes) than it does megagametophytes?

VI. POLLINATION AND FERTILIZATION

This section is included to provide continuity, and does not have demonstrations or laboratory materials provided for it.

The transfer of pollen from the anther to the stigma of the pistil is called pollination. In angiosperms this is accomplished by wind, animals, etc. After the pollen grain (immature microgametophyte) has become lodged on the stigma of the pistil, the tube cell begins to grow down the style and through the micropyle of the ovule. As the tube cell is growing the generative cell divides to form two sperm. One sperm of the mature microgametophyte unites with the egg to form a zygote and the second sperm units with the polar nuclei to form a triploid (1N + 1N + 1N = 3N) endosperm nucleus. The endosperm nucleus divides many times and eventually forms a nutritive tissue which supplies the embryo developing from the zygote with a source of food. What is meant by double fertilization in angiosperms? Do the gymnosperms have double fertilization?

VII YOUNG SPOROPHYTE

Dissect and study a soaked castor beam to find the following, but BE CAREFUL! Castor beans contain an alkaloid that is both poisonous if eaten and irritating if it gets into eyes or on sensitive tissues.

A. Embryo

The angiosperm embryo consists of three general parts, the cotyledons which are the first leaves of the new plant (buried in the middle of the endosperm of the seed), the hypocotyl, that portion of the embryo below the attachment of the cotyledons which will develop into a portion of the stem and roots, and the epicotyl, that portion of the embryo above the attachment of the cotyledons which will develop into the stem and leaves.

Two groups of angiosperms are distinguished primarily on the basis of the number of cotyledons present in the embryo. Can you name these groups and give examples of each? How many cotyledons does the castor bean have in its embryo? How many cotyledons does pine have in its embryo?

B. Seed

Angiosperm seeds which develop from ovules after fertilization, consist of an outer seed coat derived from the integuments of the ovule. The megasporangium usually has been obliterated in seeds and therefore will not be easily discernable. The castor bean possesses a great deal of endosperm whereas others like the peanut will lack endosperm entirely, because the endosperm has been digested and the food stored in the overgrown cotyledons. The food stored in endosperm is in the form of starch. How would you test for the presence or absence of starch?

C. Other seeds

Study soaked beans, corn grains and buckwheat to determine the extent of seed coat, megasporangium, endosperm and embryo.

VIII FRUIT

Since a fruit is defined as a ripened ovary with its contents, it should be clear to you that a fruit is really three generations: the parent sporophyte, the gametophyte, and the young sporophyte with its endosperm.

Following fertilization of the ovary the pistil develops into a fruit at the same time the zygote is developing into an embryo and the ovule is developing into a seed. The fruit develops from three regions of the ovary; the inner ovary wall or endocarp, the outer wall the exocarp, and the middle part of the ovary wall the mesocarp. The parts of the fruit derived from these regions of the ovary are more discernable in some kinds of fruits than others.

The following are provided as examples of fruits.

A. Olive

In the olive the endocarp is stony, the mesocarp fleshy and exocarp merely a protective skin on the outside of the fruit. Examine an olive and note these regions.

B. Buckwheat

The "seed" of the buckwheat is actually a fruit in which the one seed contained is fused to the ovary wall at one point. This kind of fruit is called an achene.

C. Other Fruits

Oftentimes there is more than one megasporophyll or carpel involved in an ovary. An ovary formed from 2 or more fused carpels is called a compound ovary. One can usually determine the number of carpels which make up a compound ovary by either counting the number of stigmas on the style or the number of cavities found in a cross section through the ovary or fruit. Is the buckwheat fruit compound? How many carpels are fused to make the compound ovary of an orange, an apple, and a cucumber?

D. Non-Fruits

All sweet foods that we call fruits are not derived from ovary tissue. For example, one section of a pineapple involves the fusion of sepals stamens, and pistils, with the receptacle and stem. The strawberry is all receptacle tissue with many hard achene fruits embedded on its outside.

Review Questions: How does the size of the angiosperm sporophyte compare with the size of the angiosperm gametophyte?
How does the size and complexity of the angiosperm gametophyte compare with the size and complexity of the moss gametophyte?
How does the size and complexity of the angiosperm sporophyte compare with the size and complexity of the moss sporophyte?
What are the major differences between the angiosperms and gymnosperms?

LABORATORY WEEK 20
GENETICS

This week's work includes the study of a genetic cross in Drosophila melanogaster, carried through two generations. Since each generation in this insect takes approximately two weeks from egg to egg, and since at least one week is required for the gathering of data, it is essential that this work be started at least five weeks early. You will be given a culture vial that contains a cross already made, and you will be expected to follow the directions for growth and further crosses when your particular flies are ready.

POSITIVE NOTICE: SINCE ETHER IS USED IN ANAESTHETIZING THE FLIES, AND SINCE ETHER IN THE AIR IS EXPLOSIVE, FLAMES AND SMOKING IN THE LABORATORY ARE ABSOLUTELY FORBIDDEN FOR THE REMAINDER OF THE TERM.

You should have a copy of the following pamphlet available for reference, but since you will use it only briefly, you need not own one yourself: Demerec, M. and B.P. Kaufmann, Drosophila Guide, edition 7, 1964.

I. THE DROSOPHILA CULTURE

A. What it is.

A few days before you received this culture, approximately three males from one pure strain of flies were put into the vial with about three virgin females from a different pure strain of flies. These flies are to be referred to as the parental (P_1) generation. The males have fertilized the females, the females have laid eggs, and larvae have probably hatched out and are crawling on and in the medium. At this age the larvae are probably too small to see, but in a few days their black jaws can be observed working in the medium.

A notation on the outside of the vial will tell you exactly what was put in the vial. Record this information in your data book, exactly as it is written, in case the writing is rubbed off the vial.

You need do nothing more with the culture until pupae are formed. See Drosophila Guide pages 3-6 for descriptions and pictures of the eggs, larvae and pupae. Keep the culture vial in your locker, and check its progress every few days. These flies are extremely sensitive to ether, so make sure that you do not keep an anaesthetizer or any trace of ether in the locker with the culture.

Refer to "Monohybride Crosses", pages 33-35 of Drosophila Guide, for general procedures. Step 1 has already been carried out for you, though the mutant strain and sex may not be the same.

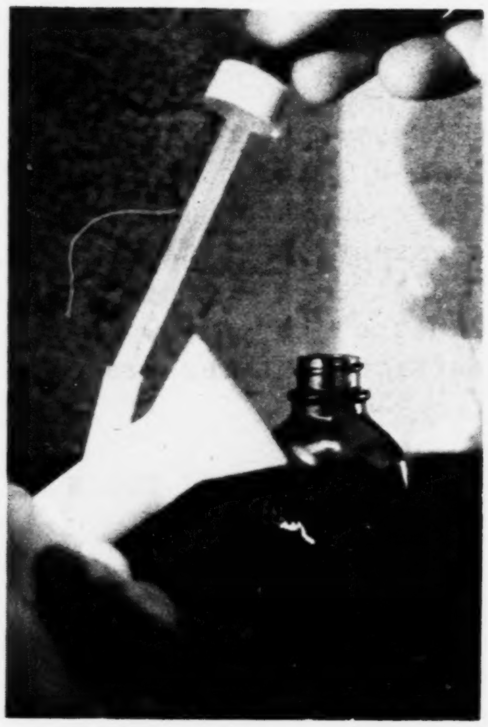


Fig. 20.1 Adding ether to the Burco anaesthetizer.

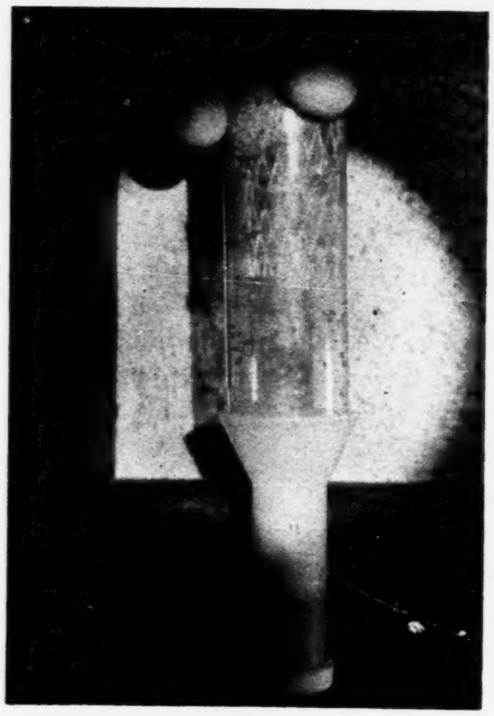


Fig. 20.2 Anaesthetizing the flies.

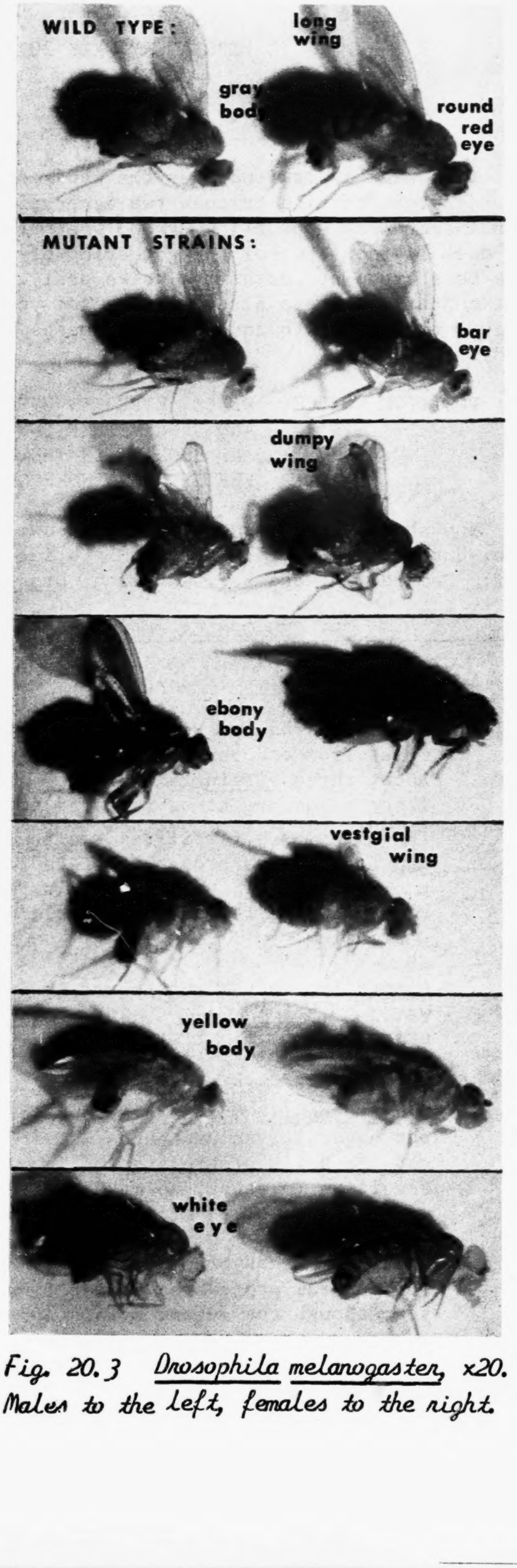

WILD TYPE:
long wing
gray body
round red eye
MUTANT STRAINS:
bar eye
dumpy wing
ebony body
vestgial wing
yellow body
white eye

Fig. 20.3 Drosophila melanogaster, x20. Males to the left, females to the right.

B. Removing and Examining the P_1 Generation

When you can observe several dozen pupa cases attached to the wall of the vial, it is time to remove and examine the parents of the cross. If you leave this step too long, members of the F_1 generation will emerge from the pupa cases and you will not be able to tell which ones were the parents.

To remove the parents, use the Burco anaesthetizer that is kept out in the air on your laboratory table. If the cotton in the anaesthetizer feels dry, add a dropper-ful of ether through the side-arm. Tap the base of the vial on the table to knock the flies down, then quickly remove the stopper and invert it over the funnel. Hold the vial and the funnel tightly together, and tap the base of the anaesthetizer on the table to knock the flies down. Make sure that the flies are all out of the vial, and then replace the stopper. Since these flies are to be killed, leave them in the anaesthetizer for 3 or 4 minutes. In E below you will want only to stun some members of the F_1 generation, and for this you should only leave the flies a few seconds.

Put the parents onto a white card or paper, and examine with a binocular microscope. Refer to Drosophila Guide pages 6-9 for the structure of the adult flies. Be absolutely certain that you can distinguish males from females quickly and accurately. Also be sure that you know exactly what your mutant strain looks like.

C. Identifying Your Mutant Strain

Each one of the cultures used contains a cross between a pure wild-type strain and a pure mutant strain. The wild type is always abbreviated "+". The mutants used are as follows.

Mutant is:	Its wild (+) allele is:
B=bar --- eyes are narrow.	Eyes are round
dp=dumpy --- wings are shorter than body.	Wings are longer than body.
e=ebony --- body is dark or black.	Body is gray
vg=vestigial --- wings are very tiny.	Wings are longer than body..
w=white --- eyes are white	Eyes are red
y=yellow --- body is yellow	Body is gray

All the males in your cross are of one kind, and all the females are of another. You can therefore become familiar with the characteristics of one sex of your mutant strain, and the other sex of the wild type. Use the camel's hair brush to move and turn the flies, so you can see all their parts. If they begin to show signs of life, put the funnel end of the anaesthetizer over them for a minute or two. Make good records, and for insurance put the flies away in a dry place where they will not be crushed. You may want to look at them again.

D. Examining the F_1

When the pupae begin to turn dark, the adults are nearly ready to emerge. Carry out the following after a dozen or more F_1 adults have emerged, as late as possible on some afternoon when you can come back to the laboratory the following morning.

Remove and kill all the emerged F_1 adults. Examine both males and females, and compare them with your records of the P_1. You should now be able to tell whether or not your mutation is dominant or recessive. In most of the crosses, all of the F_1 individuals will be wild type, though in a few crosses there will be a difference in eyes or body color between the males and females. The genetic principles involved may not yet be clear to you, so you should keep good records of the F_1 appearance. You may also want to save the flies.

E. Making a Back Cross

A back cross is defined as a cross between a hybrid and an individual like its recessive parent. It is a very useful kind of cross, yielding much information. To make this cross, you must have virgin females (see Drosophila Guide pages 15-16). Flies do not usually emerge from their pupa cases during the night, so since you removed all F_1 from your vial yesterday afternoon, any females you now have should be virgin throughout this day.

Prepare a vial of Instant Medium, following the directions on the jar. Remove the F_1 adults from their vial, etherizing them for the shortest possible time, and isolate the females. Lay the new vial on its side and place the females inside the neck of the vial. If they fall into the medium while stunned, they will die.

Then obtain about three males from the stock culture of your mutant strain. Put them in the new vial with the virgin female hybrids. Do not stand the vial upwards until all flies are fully active.

When pupae appear in this vial, remove the adults, and when the offspring appear, determine their phenotypes and proportions.

F. Making an F_2 Cross

The females in this cross need not be virgin, but it does not matter if they are. Put about three male and three female F_1 into a new vial of medium. When pupae appear, remove the adults and when the F_2 adults appear, determine their phenotypes and proportions. You will be dealing statistically with these data in II, C below.

Fig. 20.4
Table of X^2.

Choose the "degrees of freedom" column that is one less than your number of classes. Read across in that column until you come to the figure closest to your computed X^2 value. Follow that column upward and read the probability (P). If the probability is .05 or smaller, then the results you are observing are due to something other than chance.

Degrees of freedom	P=.99	.98	.95	.90	.80	.70	.50	.30	.20	.10	.05	.02	.01
1	0.0002	0.0006	0.004	0.016	0.064	0.148	0.455	1.074	1.642	2.706	3.841	5.412	6.635
2	0.0200	0.0400	0.103	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	7.824	9.210
3	0.115	0.185	0.352	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.816	9.837	11.345
4	0.297	0.429	0.711	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	11.668	13.277
5	0.554	0.752	1.145	1.610	2.343	3.000	4.351	6.064	7.289	9.236	11.070	13.388	15.086
6	0.872	1.134	1.635	2.204	3.070	3.828	5.348	7.231	8.558	10.645	12.592	15.033	16.812
7	1.239	1.564	2.167	2.833	3.822	4.671	6.346	8.383	9.803	12.017	14.067	16.622	18.475
8	1.646	2.032	2.733	3.490	4.594	5.527	7.344	9.524	11.030	13.362	15.507	18.168	20.090
9	2.088	2.532	3.325	4.168	5.380	6.393	8.343	10.656	12.242	14.684	16.919	19.679	21.666
10	2.558	3.059	3.940	4.865	6.179	7.267	9.342	11.781	13.442	15.987	18.307	21.161	23.209

II. STATISTICS OF GENETICS

Most genetic crosses are made to check a theory about the inheritance of the characteristics involved in the cross. After the data are gathered about the offspring, it is necessary to have some means to check to see if the data fit the theoretical, or expected, number of offspring. One common method of checking is to use a X^2 (Chi-square) table.

A. Learning to Use the X^2

1. Coin

Flip a coin 100 times, and record the data under Heads or Tails. What do you expect to get? 50 heads and 50 tails. Why? Because the coin has only two faces, so if the coin is not weighted in any way, you expect Heads 1 time out of 2, and Tails 1 time out of 2. This is a ratio of ½ for each of the faces. This ½ ratio can be expressed as 50% or as 0.50 x 100 = 50. For only two equal probabilities this explanation seems unnecessarily detailed, but it is necessary for later work with more than two classes of answers. To proceed with X^2 , it is necessary to determine the deviation in each class by subtracting the Expected from the Observed, then squaring the deviation, then getting the square of the deviation per unit of expected, and entering the X^2 table with this figure. Note that this is a traditional method, and you need not understand it fully in order to use it. Suppose in your experiment you obtained 44 heads and 56 tails. Then proceed as follows:

	Heads	Tails	Total
Observed (o)	44	56	100
Expected (e)	50	50	100
Deviation (o-e)	-6	+6	0
Deviation squared $(o-e)^2$	36	36	
Deviation squared per unit expected $\frac{(o-e)^2}{e}$	$\frac{36}{50} = .72$	$\frac{36}{50} = .72$	$1.44 = X^2$

Enter the X^2 Table with the figure of 1.44. Use the horizontal column of 1 degree of freedom (always enter the column that is one less than the number of classes in your problem). Read right until you come close to the figure of 1.44; in this case it will lie between 1.074 and 1.642. These numbers are in the vertical columns headed by the probability (P) values of .30 and .20. This indicates that, if we repeated this kind of experimentation for an indefinitely long time, we would obtain a deviation of this kind in 30% to 20% of the experiments, purely as a matter of chance. Obtaining a P value this large shows that our theory and our observations are a good fit -- in fact, we would expect any X^2 value to have occurred by chance alone if its probability (P) is greater than .05.

Compute the X^2 value for your own observations, and determine P.

2. Two Colors of Beans

A dish is provided on your laboratory table that contains 100 blue beans and 100 white beans. Without looking, withdraw 2 beans and record their colors, then replace them and draw again. Obtain 100 separate data, recorded as blue-blue, blue-white, white-white.

What do you expect? 25 blue-blue, 50 blue-white, 25 white-white. Why ?Because, although you have only three classes of answers, there are four possible combinations, and each of the combinations may be expected 1 chance in 4, or a ratio of 1/4 = ¼ = .25 or 25% of the time:

If one bean is:	The other bean is:	And the chance for the pair is:
Blue (0.5)	Blue (0.5)	Blue-Blue (0.5 x 0.5 = 0.25)
	White (.05)	Blue-White (0.5 x 0.5 = 0.25)
White (0.5)	Blue (0.5)	White-Blue (0.5 x 0.5 = 0.25)
	White (0.5)	White-White (0.5 x 0.5 = 0.25)

Notice the following items: The probability of any two beans forming a particular combination is equal to the probabilities of the individual bean probabilities, multiplied together. Also, when two beans are drawn at the same time, you cannot distinguish between blue-white and white-blue, and therefore you must consider these two to be a single class.

Suppose in your experiment you obtained 20 blue-blue, 45 blue-white, and 35 white-white. Then proceed as follows:

	BB	BW	WW	Total
Observed (o)	20	45	35	100
Expected (e)	25	50	25	100
Deviation (o-e)	-5	-5	+10	0
$(o-e)^2$	25	25	100	
$\frac{(o-e)^2}{e}$	$\frac{25}{25}$ = 1.0	$\frac{25}{25}$ = 0.5	$\frac{100}{25}$ = 4.0	5.5 = X^2

Enter the X^2 Table with figure 5.5. Which Degree of Freedom will you use? Make sure you know this before proceeding! Since there are three classes, use two degrees of freedom. The X^2 value of 5.5 will lie between 4.605 and 5.991, making the P value lie between .10 and .05. Is this a good fit of the data to the theory?

Compute the X^2 value for your own observations, and determine P.

B. Applying the X^2 To Parent Determination in Corn

In corn, purple (P) is dominant to white (p); and smooth (S) is dominant to shrunken (s). In the special ears of corn provided in the laboratory, care has been taken to make sure that each entire ear is the offspring of only two parents, by bagging the silks as they appeared and pollinating them only with pollen from a single plant. Therefore the ratios of purple to white, and smooth to shrunken, will tell you something (sometimes everything) about the genotypes of the parents. Before proceeding, determine the phenotypes of the following sets of parents, and the genotypes and phenotypes of the offspring:

PP x PP	SS x SS	PPSS x PPSS
pp x pp	ss x ss	ppss x ppss
PP x pp	SS x ss	PPSS x ppss
Pp x pp	Ss x ss	PpSs x ppss
Pp x Pp	Ss x Ss	PpSs x PpSs
etc.	etc.	etc.

Choose one of the ears of corn provided in the laboratory. Do not remove the kernels from it! Count the various classes of kernels that you find on it, and then formulate a theory as to what the two parents might have been.

For example, you might obtain 178 purple and 163 white, which looks as though it might be ½ purple and ½ white, the result of Pp x pp cross. If so, you would have expected 170.5 of each kind of kernel, rather than what you observed. Use 170 and 171 as the *e* figures, since it would be nonsense to expect half a kernel. Work out the X^2 to see if your theory fits the observed data. If not, try other theories until you come up with the one that is the best fit to the data.

C. Applying X^2 to Drosophila Crosses.

In these cases, you have been collecting the observed data (*o*) for some time, but now you need to know what you might have expected. You will therefore need to decide whether your mutant gene was dominant, recessive or non-dominant; and you will need also to know whether it was sex-linked or autosomal; before you can compute the expected (*e*) ratio. You may already have decided this, but if not then the following guide will help. In the following, the symbol + refers to the wild allele, m refers to a recessive mutant, M refers to a dominant mutant, and y to the y-chromosome which carries no allele at all. You may substitute your own symbolism (dp, vg, etc.) for that used.

1. If the mutant is a recessive autosomal characteristic.

P_1 cross: male ++ wild X female mm mutant (same if sexes reversed)
Gametes : (all +) (All m)
F_1 : all +m wild

F_1 to F_2 cross: male +m wild X female +m wild (same if sexes reversed)
Gametes : (½+ and ½m) X (½+ and ½m)

F_2 :

	½ +	½m
½ +	¼ ++ wild	¼ + m wild
½ m	¼ +m wild	¼ mm mutant

(three-fourths wild and one-fourth mutant, regardless of sex)

Backcross : male mm mutant X female F_1 +m wild
Gametes: (all m) ½ + and ½ m)

Offspring :

	½ +	½ m
m	½ +m wild	½ mm mutant

(half wild and half mutant regardless of sex)

2. If the mutant is a recessive sex-linked characteristic , and the female carried the mutant gene.

P_1 cross: male +y wild X female mm mutant
Gametes: (½ + and ½ m) (all m)

F_1 :

	m
½+	½ +m wild females
½ y	½ my mutant males

F_1 to F_2 cross: male my mutant X female +m wild

Gametes : (½m and ½y) (½+ and ½m)

F_2:	½ +	½ m
½ m	¼ +m wild females	¼ mm mutant females
½ y	¼ +y wild males	¼ my mutant males

Backcross: male my mutant X female F_1 +m wild
Gametes: (½m and ½y) (½ + and ½m)

Offspring:

	½+	½m
½m	¼ +m wild females	¼mm mutant females
½y	¼ +y wild males	¼my mutant males

3. Work through other possibilities of dominance, sex linkage, etc., until you obtain an expected ratio that fits your results well by inspection. Choose this one, and apply the X^2 to your results.

4. Write a report on your Drosophila cross. .